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Effects of exogenous enzymes and direct-fed microbial on broiler performance and nutrient digestibility when fed variable inclusions of soy products

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Effects of exogenous enzymes and direct-fed microbial on broiler performance and nutrient digestibility when fed variable inclusions of soy products

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DEDICATION

To my loving parents for their encouragement and for showing me anything is possible with hard work. To my supportive brother, your light-hearted way of looking at life helped ground me. To my grandparents, for your constant love and care. To A. J., you have given me nothing but love and support throughout this process. To my constant canine companion, Cy, you have helped see me through it all. Without the love and support of all my family and friends this journey would not be possible.

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ABSTRACT

The objective was to examine the effects of feed additive combinations, including xylanase, amylase, and protease enzyme (XAP), with β-mannanase (MAN) or direct-fed microbial (DFM) supplementation on broiler performance and nitrogen corrected apparent metabolizable energy (AMEn) when fed diets containing various soy products concentrations. Two experiments were conducted from 10 to 21 d of age by feeding three soy product inclusions of low (20%), intermediate (28%), and high (35%). Feed additive combinations were supplemented across the three concentrations of dietary soy in both experiments. In experiment 1 there was no enzyme supplemented, XAP (100 g/MT) alone, and XAP with MAN (100 g/MT or 50 g/MT). In experiment 2 there was no supplementation, XAP (100 g/MT) alone, and XAP with DFM (100 g/MT or 50 g/MT). Experiment 1 resulted in reduced feed intake ($P \leq 0.01$) and increased body weight gain (BWG) ($P \leq 0.10$) with intermediate and high soy compared to low soy inclusion. Supplementation with all enzyme combinations decreased feed intake ($P \leq 0.01$) compared to non-supplemented diets and improved feed efficiency (FE; $P = 0.02$) and feed conversion ratio (FCR; $P = 0.01$). There was an interaction between soy inclusion and enzyme supplementation as XAP+½MAN improved AMEn ($P = 0.05$) in high soy treatments. In experiment 2, there was an interaction as the intermediate soy diet with XAP and DFM decreased BWG ($P = 0.02$) compared to all other soy combinations, no treatment differed from control diets without enzyme. Feed intake was not different, and high soy improved FE and FCR compared to low and intermediate soy ($P \leq 0.05$). Enzyme supplementation with XAP alone improved FE and FCR over the combination of XAP and DFM ($P \leq 0.05$). Low soy decreased AMEn ($P \leq 0.01$) compared to intermediate and high soy. Overall, high soy diets out-performed low soy treatments possibly

due to increased fiber in low soy diets from DDGS and canola meal ingredients. In experiment 1, FE and FCR were improved with all combinations of enzyme and AMEn of high soy diets was increased with enzyme supplementation demonstrating a positive effect of enzyme addition. In experiment 2, XAP and DFM did not improve broiler performance, possibly due to a well-established microbial population in the gastrointestinal tract by the time feed additive supplementation occurred.

Keywords: XAP, β-mannanase, direct-fed microbial, broiler, AMEn

CHAPTER 1. INTRODUCTION

Soybeans are grown for their oil content which is utilized for food and industrial applications. The by-product residual meal is used as a quality protein meal which is of significant demand in poultry and other livestock production systems (Asbridge, 1995; Soybean Meal Information Center, 2011). Utilization of soybean meal in poultry production is about 50% of all the soybean meal used in livestock production with swine, at 26% of total soybean meal use resulting in non-ruminant production using over 75% of the soybean meal produced in the United States (Soybean Meal Information Center, 2011). Even though soy protein is ubiquitous in poultry rations, anti-nutrients present can decrease the overall feeding value of soy (Marsman et al., 1997; Kocher et al., 2003).

Haemagglutinins (lectins), saponins, pectins, non-starch polysaccharides (NSP), trypsin inhibitor, phytates, raffinose, stachiose, and β-mannans are some of the main anti-nutrients found in soy (Marsman et al., 1997; Kocher et al., 2003). Processing methods can eliminate a portion of these anti-nutrients, but several remain (Perkins, 1995; Marsman et al., 1997). Many anti-nutritional components in soy products and other cereal grains come in the form of carbohydrates that are indigestible or lowly digestible. Soybean meal contains 15% starch and sugars, a portion of which are not digestible, and 16-20% insoluble NSP, the majority being low or not digestible (National Research Council 1994; Bach Knudsen, 1997).

Monogastrics lack endogenous enzymes to break down soybean anti-nutrients (Hessing et al., 1995; Joergensen et al., 1996), resulting in digestive inefficiency (Choct et al., 2010). Poor broiler growth performance and nutrient digestibility have been observed when fed diets containing soybean meal as the primary protein source (Irish and Balnave, 1993; Marsman et al., 1997; Kocher et al., 2003). Other young monogastrics, such as swine,

have also responded to soybean meal, as the primary protein source, with reduced growth performance (Li et al., 1991; Friesen et al., 1993). In general, pigs can digest NSP and other anti-nutrients better than chickens due to a better fermentative capacity in the large intestine and a longer digesta transit time in the large intestine (Choct and Cadogan, 2001). Soybean meal is a quality protein source however it tends to be low in energy (National Research Council, 1994), and therefore the opportunity to increase the feeding value of soy through use of feed additive supplementation exists (Cowieson et al., 2010; Zanella et al.,1999). Rising cost of soybeans and corn in the Midwest has placed additional strain on increasing energy utilization from these common feed ingredients.

Non-nutritive feed additives such as exogenous enzymes and direct-fed microbials are a means to overcome the anti-nutritional components of soy products by improving bird performance (Bedford, 1996; Zanella et al., 1999; Bach Knudsen, 1997), overall nutrient availability (Burnett, 1996), and potentially immune and intestinal health (Zou et al., 2006). Increasing economic cost, continued popularity of soy products in poultry rations due to their well-balanced amino acid profile and high protein content, and use of lower quality feed ingredients due to decreased availability have prompted feed additive research. Insoluble NSP are highly resistant to digestion and are primarily composed of arabinose, xylose, mannose, galactose, and cellulose (Bach Knudsen, 1997). Pectinase, cellulase, β-mannanase, xylanase, α-amylase, and β-glucanase, are some of the exogenous enzymes utilized in diets containing soybean meal to potentially alleviate such anti-nutrients by hydrolyzing NSP and maximizing nutrient utilization (Bach Knudsen 1997; Kocher et al., 2003).

Exogenous enzyme products operate by increasing availability and retention of nutrients already present in feedstuffs (Marsman et al., 1997; Zanella et al., 1999; Kocher et

al., 2003). Supplementation of xylanase, amylase, and protease enzymes are used to target NSP, starch, and protein consequently improving digestion in monogastric animals fed corn-soybean meal diets (Burnett, 1996). Breakdown of plant cell wall carbohydrates by NSP enzymes can eventually lead to production of short-chain oligosaccharides (di- and tri-saccharides). These oligosaccharides are known to be a substrate for bacterial fermentation, potentially positively altering bacterial populations within the gut (Fuller, 2001; Choct et al., 2010). It is thought that β-mannanase has three primary modes of action against β-mannans, these mechanisms include decreased viscosity, improved energy metabolism, and reduced innate immune stimulation (Jackson et al., 2004). Research has shown exogenous enzymes can increase the feeding value of soy and other grain products, when fed to broilers, leading to increased growth performance and efficiency (Cowieson et al., 2010; Zanella et al.,1999). Exogenous enzyme use in soy based diets is focused on complementing and increasing endogenous enzyme activity along with targeting a wider group of substrates than monogastrics are capable of targeting endogenously (Cowieson et al., 2010).

Probiotics are marketed as direct-fed microbials (DFM), and are commonly strains of yeasts and fungi (Fuller, 2001). Use of DFM can reduce incidence of enteric disease challenge and improve health status of an animal by maintaining a beneficial microbial population (Fuller, 2001; Lee et al., 2010). These and other gut health benefits stem from the process of competitive exclusion of pathogenic bacteria (Nurmi and Rantala, 1973; Schneitz, 2005), resulting in improved immune characteristics through stabilization of intestinal barrier function and protection against entero-pathogens (Salminen et al., 1996; Schneitz, 2005). It has also been shown that DFM play a significant role in meeting bird energy requirements through regulation of hind gut fermentation and synthesis of SCFA (Caballero-Franco et al.,

2007), claims of bird performance enhancement by DFM have also been made (Corrier et al., 1995; Jin et al., 1998).

Presence of anti-nutritional factors in soy products can present a challenge when fed in higher concentrations to monogastric animals. Some anti-nutrient properties can be eliminated or reduced through further processing methods such as heat treatment, while others like NSP, oligosaccharides, and phytates are targeted through supplementation of feed additives. Carbohydrate targeting enzymes commonly utilized include β-mannanases and xylanases. Though research has demonstrated that positive impacts on bird performance and nutrient digestibility can result from supplementing feed additives, further experimentation determining interactions when combinations of exogenous enzymes and DFM are fed to broilers in soy based diets is of importance. Therefore the objective of this thesis research is to examine the effects of feeding a multi-enzyme (XAP) in combination with either a β-mannanase or DFM on broiler growth performance and nutrient digestibility when fed three concentrations of dietary soy including soybean meal and toasted full fat soy ingredients.

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CHAPTER 2. LITERATURE REVIEW

Soy products

Usage and processing

Soybeans are the leading oilseed produced in the United States and worldwide, making up about 90% of all domestic oilseed production (USDA ERS, 2014). Due to market demand, soybeans are grown mainly for their oil content and utilized in food or industrial applications; however the high quality protein meal produced is of significant demand in animal production (Figure 2.1) (Asbridge, 1995; USDA ERS, 2014). Production of soybeans in the United States is predicted to be 3,289 million bushels for 2013/2014 with an estimated 20,005 tons of soybean meal produced from those soybeans (USDA ERS, 2014).

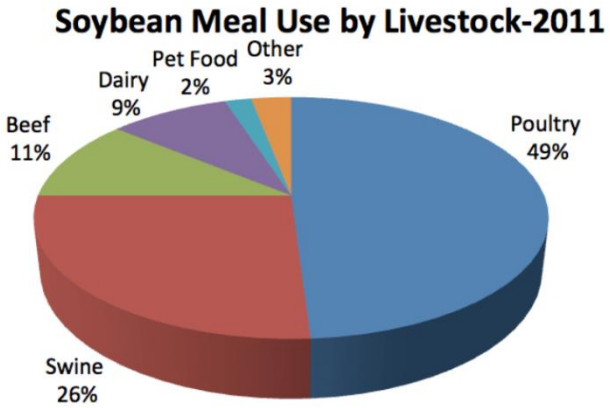

Figure 2.1. Pie chart demonstrating utilization of soybean meal in livestock production, most notably in poultry. Adapted from Soybean Meal Information Center, 2011.

In general, raw soybeans have decreased feeding value compared to processed soy products. Comparison of raw and roasted soybeans shows heat treatment is a valuable tool in increasing feeding value of soy products. Information based on 100 g portions reveals raw soybeans contain 36.5% protein, 19.9% fat, 30.2% carbohydrate, and 5.0% crude fiber.

Analysis of roasted soybeans showed 35.2% protein, 25.4% fat, 33.6% carbohydrate, and 4.6% crude fiber (Perkins, 1995). Processing of soybeans into meal form is often accomplished by mechanical and solvent extraction. Mechanical methods use continuous screw presses (expellers) to compress the soybeans into cake form, but are not commonly utilized due to incomplete oil extraction. Solvent extraction is more common and uses hexane to recover oil product from soybeans; the three main steps in this process are preparation, extraction, and desolventing or toasting. During the preparation step of solvent extraction the decision to keep or remove soy hulls is made, therefore leading to production of one of two common soybean meals sold to the animal industry, soybean meal 44, named due to an addition of soy hulls resulting in a total protein content of 44% or soybean meal 48, named due to no addition, or limited addition of soy hulls resulting in 48% crude protein. Soybean processing methods are quite efficient and continue to evolve with time.

Nutrient profile

Traditionally soy has been utilized in poultry and other monogastric diets within the United States because it is considered a high quality protein source. Nutritionally soy is a good source of protein with a fairly well-balanced amino acid profile, although it is low in methionine, the first limiting amino acid in poultry diets (Centeno et al., 2006). Unlike other plant protein sources soy has greater lysine content (Table 2.1) (McNaughton et al., 1981).

Table 2.1. Amino acid profile of soybean meal compared to other plant protein sources. Adapted from National Research Council (1994).

Amino Acid (%)	Ingredient			
	Soybean Meal 48	**Sunflower Meal**	**Corn Grain**	**Wheat Bran Flour by-product**
Lysine	2.96	1.24	0.26	0.59
Methionine	0.67	0.80	0.18	0.23
Threonine	1.87	1.29	0.29	0.50
Cysteine	0.72	0.64	0.18	0.37

Broiler type birds require 0.50% methionine per kg of diet in the first 0 to 3 wk and soybean meal typically provides 0.62 to 0.67% methionine depending of the amount of hull added back into the product (National Research Council, 1994). Whole soybeans contain about 35% carbohydrates originating from the hull (92%), hypocotyl (43%), and cotyledon (29%) structures, 4.7% crude fiber, 17.8% fat, less than 1% starch, and 37.9% protein (Perkins, 1995). Soybean seeds, meal solvent extracted with hulls contain about 44% protein and 7.0% crude fiber whereas without hulls soybean meal contains 48.5% protein and only 3.9% crude fiber, both contain minimal amounts of fat at 0.5% (National Research Council, 1994). Dry matter and energy digestibility are relatively low, along with 10 to 15% of amino acids being indigestible (Pierson et al., 1980).

Soybean meal contains 35% carbohydrates (Figure 2.2) and of that 10% is free sugar consisting of 5% sucrose, 4% stachyose, and 1% rafinose (Karr-Lilienthal et al., 2005). Carbohydrate content of soybeans from separate geographic regions fluctuates leading to the variable nature of soybean meal (Parsons et al., 2000; Karr-Lilienthal et al., 2005). It has been hypothesized that the rather under-developed gastrointestinal tracts of young monogastics are not able to tolerate oligosaccharides well (Bach Knudsen, 1997; Douglas et al., 2000). Therefore it is important to explore enzyme options to reduce the anti-nutritional impacts of soy products.

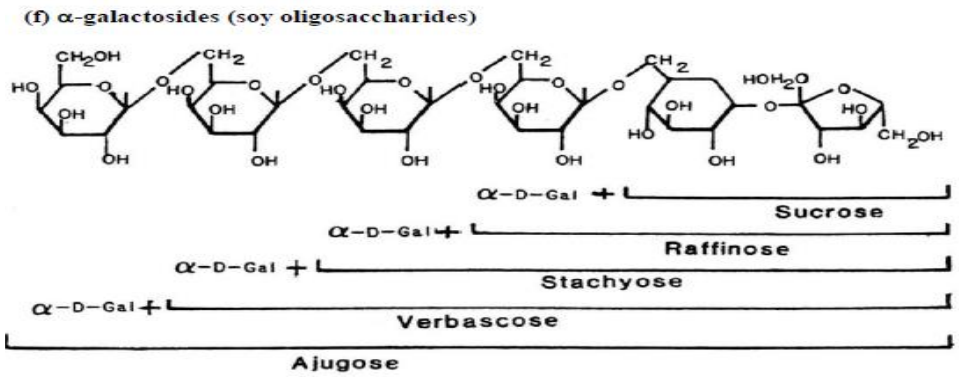

Figure 2.2. Chemical structures of soy carbohydrates. Choct et al. (2010).

Most commonly, high protein soybean meal is used in broiler rations. High protein soybean meal, also known as dehulled soybean meal, contains NSP in the form of pectic polysaccharides. 1,4-β-arabinogalactans and 1,2-1,4-β-rhamnogalacturonans are the two major forms of pectic polysaccharides, or soy pectin, in dehulled soybean meal. Galactose is the predominant sugar found in fiber of dehulled soybean meal (0.142% hulls, 0.547% meal) while arabinose (0.278% hulls, 0.264% meal), mannose/xylose (0.455% hulls, 0.142% meal) are sugars found primarily in soy hulls (Vahjen et al., 2005). Soybean hulls also have a high content of cellulose and hemicellulose (Perkins, 1995).

Addition of soy hulls into poultry rations can result in decreased energy availability and digestibility of nutrients. Digestibility of some amino acids has been shown to be reduced with inclusion of soy hulls in swine diets (Dilger et al., 2004). Although soy is desirable due to its high protein content and favorable amino acid composition, the dietary energy value of soybean meal is low and tends to be inconsistent (Daenicke et al., 2001). Therefore potential to improve feeding value of soy through exogenous enzyme addition is an important area of research. Factors including variety of soy cultivated, regional variation of soy, as well as processing conditions such as heat treatment or solvent extraction can cause variation in the nutrient availability of soy products (Douglas et al., 2000). Enzyme addition

has potential to reduce some of the variation in soybean meal that occurs due to processing and soy cultivar.

Anti-nutrients in soy products

Haemagglutinins (lectins), saponins, pectins, non-starch polysaccharides (NSP), trypsin inhibitor, phytates, raffinose, stachiose, and β-mannans are some of the main anti-nutrients in soy (Marsman et al., 1997; Kocher et al., 2003). Heat treatment and other processing methods are able to eliminate portions of anti-nutritional factors, such as trypsin inhibitor and lectins, although these components are not completely purged (Erickson, 1995; Kocher et al., 2003). Monogastrics lack sufficient quantities of endogenous enzymes that enable them to break down anti-nutrients such as trypsin inhibitor and NSP (Hessing et al., 1995) and can result in digestive inefficiencies (Irish and Balnave, 1993; Marsman et al., 1997; Kocher et al., 2003). Another important consideration is differences in the digestive tract between monogastrics, as passage rate in swine is approximately 12 to 24 h compared to 2 to 4 h in poultry. Reduced passage time results in decreased fermentation capacity that could potentially degrade anti-nutrient factors. Therefore anti-nutrients often impact poultry to a greater extent than other monogastrics. (Entringer et al., 1975; Mateos et al., 1982).

Lectins, saponins, pectins, and phytates

A main function of lectins in soybeans is to act symbiotically with rhizobium bacteria in roots to provide the plant nourishment (Sharon and Lis, 1972; Daniel, 2005). Lectin is one protein whose anti-nutrient activity in soy resists destruction by endogenous enzymes, although reduction during processing into soybean meal can be successful (Perkins, 1995;

Daniel, 2005). It has been shown that lectin activity is not always completely eliminated and can cause diminished performance in animals fed diets containing soybean meal. Lectins react with the carbohydrates in cellular membranes and can damage cellular components. If damage from lectin activity accumulates, negative impacts on the animal's gastrointestinal tract and immune function can result. When consumed in large quantities lectins can impact the overall function of an animal's gastrointestinal tract including secretory, absorptive, protective, and digestive mechanisms (Sharon and Lis, 1972; Daniel, 2005).

Saponins (Figure 2.3) are another class of anti-nutrients present in soy and are a type of glycoside widely distributed in plants (Berhow et al., 2002). It has been observed that saponins interact with the mucosa in the gastrointestinal tract of animals by attaching to the cellular membrane causing increased cellular permeability to the lumen environment. This increase in permeability could potentially leave enterocytes exposed to pathogenic infiltration (Berhow et al., 2002). Unlike other soy anti-nutritional components, saponins are not eliminated through heat treatment, only alcohol extraction is capable of removing saponins (Erickson, 1995).

Pectins are another anti-nutrient present in soy products. Polygalacturonase, also known as pectinase, is utilized to hydrolyze pectins (Kocher et al., 2003). Pectin is a complex polysaccharide associated with plant cell walls consisting of an alpha 1-4 linked polygalacturonic acid backbone with rhamnose residues and modified with neutral sugar side chains and non-sugar components like acetyl, methyl, and ferulic acid groups (Williams and Phillips, 2008). Phytates, another anti-nutrient, act by binding to nutrients such as calcium, zinc, and iron rendering them unavailable for animal growth (O'Dell, 1969). Breakdown of phytates into an available phosphorus source results in less capability of phytate to bind

minerals, starch, and proteins (Selle and Ravindran, 2007). Phytase is a well-developed commercially used enzyme that targets phytate. Since this enzyme is out of the scope of my thesis work I will not detail the negative effects of soy phytate on poultry.

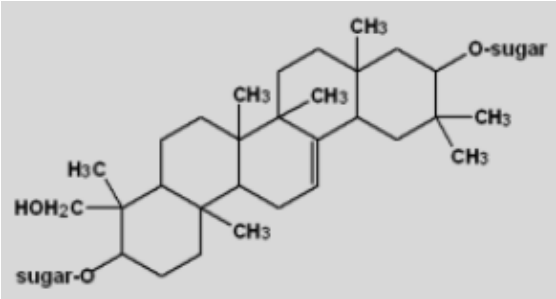

Figure 2.3. Structure of saponin. From www.phytochemicals.info.

Trypsin inhibitor

Trypsin is an enzyme which is secreted by the pancreas as an inactive zymogen, trypsinogen. Trypsinogen makes up about 10% of the total digestive enzyme secreted by the pancreas (Denbow, 2000). Once released from the pancreatic ducts into the distal portion of the ascending duodenum trypsinogen is activated by the enteropeptidase enzyme to form trypsin. Activation of trypsin catalyzes the cleavage of peptide bonds and production of free amino acids. Consequently, inhibition of trypsin causes the pancreas to undergo hypertrophy due to an increase in pancreatic secretion stimulation (Green and Lyman, 1972). Trypsin inhibitors, also known as serine protease inhibitors have been found in soy and are known to interfere with the digestion of protein (Kunitz, 1947). Like lectins, trypsin inhibitor can be rendered mostly inactive through heat processing (Han et al., 1991; McNaughton et al., 1981).

Data show that when soybean meal is fed to broilers from 1 to 21 d and is appropriately heated, trypsin inhibitor is decreased and body weight, along with feed efficiency is increased. However over processing of soybean meal can cause destruction of

available amino acids resulting in depressed broiler performance (McNaughton et al., 1981). It is widely accepted that there is increased feeding value associated with properly heat treated soy products over raw soy products in monogastric diets. Under processing of soy can lead to intact trypsin inhibitor that may also limit poultry feed efficiency and weight gain (Han et al., 1991). Due to variation in soybean processing, opportunity for exogenous protease products exist. If trypsin inhibitors are not completely eliminated during processing protease enzymes will target trypsin inhibitor and other protein substrates potentially leading to improved digestibility. When heat treating soy products, it is important to consider the delicacy between under and over processing because of its strong relationship with protein quality and overall broiler performance.

Non-starch polysaccharides

Presence of indigestible carbohydrates, oligosaccharides, and non-starch polysaccharides (NSP) are important factors in determining the nutritional value of soybean meal (Kocher et al., 2003). Galactans, xylans, and mannans are forms of plant cell wall NSP typically associated with soybean meal (Centeno et al., 2006). Monogastrics do not produce endogenous enzymes capable of digesting NSP (Joergensen et al., 1996). Therefore endogenously produced digestive enzymes may have limited access to nutrients bound in cell walls rich in NSP limiting energy and nutrient digestibility of soybean meal (Bedford, 1996).

Bach Knudsen (1997) found soybean meal contains approximately 6 to 13% soluble NSP and about 8 to 15% insoluble NSP (Figure 2.4). Enzymes targeting NSP tend to have inconsistent results for diets based on soybean meal compared to those utilizing wheat and barley ingredients, due to greater concentration of insoluble NSP in soybean meal compared to the soluble NSP in wheat and barley (Bach Knudsen, 1997). Soluble NSP in soy products

can be utilized by the intestinal microflora unlike insoluble NSP that are more difficult to degrade. Soluble NSP are considered a main anti-nutrient in wheat products because soluble NSP increases digestive viscosity resulting in decreased nutrient digestibility. Although soluble NSP are not as prevalent in soy, insoluble NSP are considered primary anti-nutrients because of their energy diluting effect on soy products (Kocher et al., 2002). Total concentration and composition of NSP and crude protein (CP) can vary between soybeans processed at different time points. Analysis of twelve commercial sources of soybean meal revealed CP content varied from 41 to 51% and protein solubility varied from 71 to 83%, while amino acid levels varied in accordance with CP (Douglas, 2000).

The anti-nutritive effect of NSP on nutrient digestion is positively related to dietary NSP concentration due to limited fermentation with reduced hindgut capacity of poultry. The hindgut microbial flora of poultry possesses the capability to ferment non-digestible materials, but this is limited due to the short transit time of digesta in the gastrointestinal tract (Choct and Annison, 1990; Choct and Cadogan, 2001). Non-starch polysaccharides are capable of binding nutrients and forming complexes with digestive enzymes and some regulatory proteins in the gut (Angkanaporn et al., 1994). Viscous NSP can enhance bile acid secretion and subsequently result in significant loss of these acids in the feces of rats (Ide et al., 1989). Binding of bile salts, lipids, and cholesterol by NSP could result in increased hepatic synthesis of these products resulting in changes in the digestive and absorptive dynamics of the gut, consequently increasing maintenance requirements resulting in poor overall efficiency in nutrient assimilation by the animal (Vahouny et al., 1981).

Supplementation of diets containing 37% soybean meal with a commercial xylanase amylase protease (XAP) exogenous enzyme at 0.10% inclusion resulted in increased ileal

digestible energy of one randomly selected soybean meal source (3.076 kcal/g DM) compared to the non-supplemented corn and soybean meal reference diet (2.917 kcal/g DM). Broilers were supplemented with exogenous XAP at 7 d. Also chick growth performance was significantly affected by soybean meal source with gain:feed significantly increased for source twelve (0.701 g/g) compared to source eleven (0.644 g/g), both of which were randomly selected soybean meals supplemented with XAP (Douglas et al., 2000). Distinct soybean meal sources contain variable anti-nutrient content and nutrient digestibility that may influence the capability of exogenous enzyme sources, such as XAP, to improve diet digestibility when fed to young broilers (Bach Knudsen, 1997; Douglas et al., 2000). There is potential for enzyme supplementation to play an important role in increasing overall feeding value of soy sources used in poultry production. Degradation of nutrients and anti-nutrients alike can increase the overall energy available to the bird possibly decreasing performance variation among poultry fed soy from varying sources (Hessing et al., 1995).

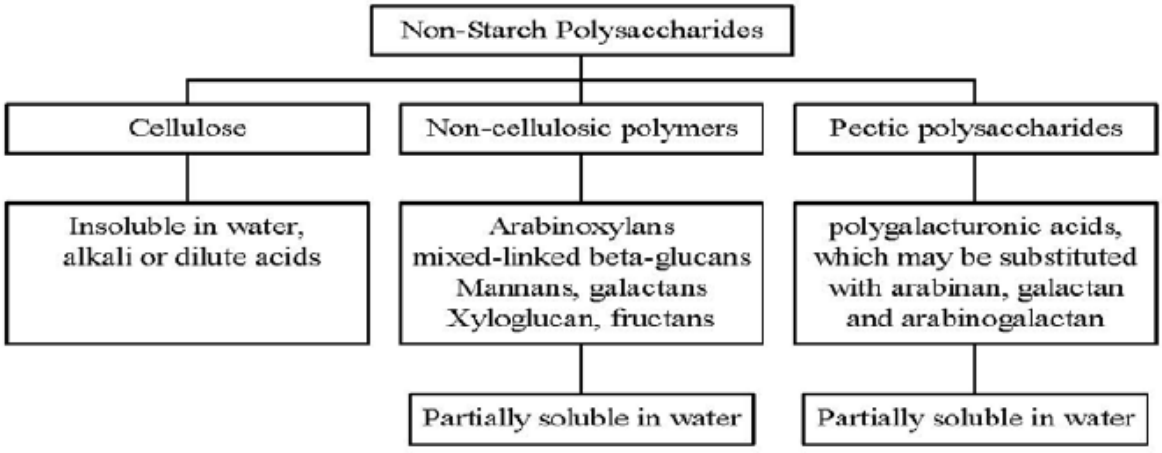

Figure 2.4. Classification of non-starch polysaccharides (NSP). From Choct et al. (2010).

Limited degradation of soy NSP occurs through chemical and microbial means and is impacted by factors such as animal age, solubility, chemical structure, and quantity in the

diet (Bach Knudsen and Hansen, 1991). For example, NSP disappearance from the gastrointestinal tract was 13% when adult cockerels were fed 6.9% NSP from defatted, de-hulled soybean meal (Carre et al., 1990). The ability to degrade NSP can differ significantly within species as it has been apparent that digestibility of corn-soybean meal NSP was significantly increased by 14.2% in adult cockerels (layer strain) compared to 3 wk old broiler chicks. Nitrogen corrected apparent metabolizable energy values were lower in broiler chickens compared to adult cockerels, with an average 191 kcal/kg DM difference resulting from bird type and age. Broilers also secreted significantly more organic acids (11.032 g/kg diet) compared to the adult cockerels (4.190 g/kg diet). Loss of organic acids relates to energy loss and overall digestive inefficiency of broiler chicks compared to adult cockerels. A potential explanation for improved ability of adult cockerels to degrade NSP may be a result of maturation of gut microflora and production of various glycanases as birds age (Carre et al., 1995). Therefore exogenous enzymes such as xylanase and β-mannanase supplemented at hatch may support the naïve gastrointestinal tract of broiler chicks.

Soy NSP can be fermented by microbes to produce short chain fatty acids such as acetate, propionate, and butyrate. Swine focused *in vitro* fermentation assays have found that after 12 h soy soluble NSP and oligosaccharides produce 2.4, 1.9, 0.7, and 5 mmol/g of OM for acetate, propionate, butyrate, and total SCFA, respectively (Smiricky-Tjardes et al., 2003). While these values are likely reduced in poultry compared to other monogastrics due to diminished hindgut fermentation capacity of poultry (Choct and Cadogan, 2001), in general production of SCFA contributes to the energy needs of the bird and are efficiently absorbed in poultry (Carre, 1995). Partial degradation of NSP in soy products is beneficial for diet utilization however higher levels of hydrolysis can lead to production of oligomers.

These end products cause anti-nutritive effects and lead to increased osmotic pressure and microbial fermentation in the small intestine negatively impacting nutrient absorption (Vahjen et al., 2005). Exogenous enzyme supplementation has potential to increase NSP degradation of soy feed ingredients (Rutherford et al., 2007; Kalmendal and Tauson, 2012).

Carbohydrase enzymes are used to target and increase degradation of NSP (Kocher et al., 2003). Research has found carbohydrase enzymes improve body weight gain, feed conversion ratio, and AME of broilers, when fed corn-soybean meal diets, possibly due to increased degradability of NSP. Carbohydrase enzymes have also been shown to increase feeding value of corn-SBM diets due to increases in ileal digestibility of CP, starch, and fat. Cell wall degrading and protease activity of enzymes can also result in increased nutrient digestibility (Marsman et al., 1997; Zanella et al., 1999). One mechanism proposed for increased AMEn of diets containing β-glucanase, hemicellulose, and pectinase enzymes is solubilization of pectic NSP which releases nutrients from cell walls of SBM products (Kocher et al., 2003).

Oligosaccharides (raffinose and stachyose)

Oligosaccharides (Figure 2.2) are found in corn and soy products and are also referred to as α-galactosides due to their chemical structure consisting of saccharose and galactose linked by α-galactosidic bonds (Choct, 1997). Corn is normally considered to have low oligosaccharide content, although actual concentrations of oligosaccharide can vary from 5 to 8% (Knudsen, 1997; Huisman et al., 2000). Concentration of oligosaccharides raffinose (0.1-1.5%) and stachyose (1-6%) of conventional soybean meal can vary depending on geographical location, harvest conditions, and post-harvest processing (Parsons et al., 2000;

Karr-Lilienthal et al., 2005). The oligosaccharide glucurono-arabinoxylans can be degraded into oligomers by exogenous enzymes (Huisman et al., 2000). In addition to naturally occurring oligomers, oligosaccharides can be broken down into oligomers and impact metabolizable energy through loss of water in the lumen by osmotic up-regulation and increased colonization of microbes in the gastrointestinal tract, leading to increased excreta output (Kocher et al., 2003).

Raffinose and stachyose are two main anti-nutrient oligosaccharides present in soybean meal (Table 2.2). Monogastrics contain no endogenous enzymes capable of digesting certain oligosaccharides like raffinose and stachyose (Veldman et al., 1993). Raffinose content is positively correlated to oil content and stachyose is positively correlated with protein meal concentrate. When raffinose and stachyose enter the gastrointestinal tract little digestion of these oligosaccharides occurs in the upper portion of the tract, most raffinose and stachyose resist digestion into the large intestine where fermentation by microbes commences. Fermentation by host microbes results in excess gas production and loss of energy leading to decreased digestive efficiency (Hymowitz et al., 1972; Zhang et al., 2001). Around 99% of sugars found in soybean seeds are sucrose along with raffinose and stachyose (Perkins, 1995). Ileal digestibility of raffinose and stachyose was low in roosters at less than 1%; however, total tract disappearance was increased to 84 to 90% (Coon et al., 1990). Others have estimated the total tract apparent digestibility was 82% for oligosaccharides in cockerels fed a diet based on soybean meal and corn starch (Carre et al., 1990).

Table 2.2. Content of carbohydrates in soybean meal. Adapted from Bach Knudsen (1997).

	Soybean Meal	
	Mean (g kg-1 DM)	SD
Number of Samples	6	
Monosaccharides	7	1
Raffinose	10	1
Stachyose	47	4
Verbascose	3	2
Xylose	17	3
Mannose	8	2
Galactose	16	3
Arabinose	9	2
Cellulose	62	18
Total NSP	217	27

Research examining the prebiotic effect of oligosaccharides has reported a change in the number and population of the gut micro flora, possibly indicating a beneficial effect. Broilers were challenged with *S. dublin* from 3 to 10 d of age while receiving 4,000 ppm of dietary mannanoligosaccharide. The number of birds testing salmonella positive at d 10 was significantly less when supplemented with mannanoligosaccharide 90 versus 56% (Spring et al., 2000). Others examining performance parameters related to elevated levels of oligosaccharides in diets argue that increases in fluid retention, hydrogen production, and diarrhea may result causing inefficiencies related to nutrient utilization (Saini, 1989; Coon et al., 1990).

Soy is a high quality source of proteins, although anti-nutrient factors and added fiber components, such as hulls, limit the energy available for metabolism in poultry. Therefore there is tremendous opportunity for non-nutritive feed additives such as exogenous enzyme and direct-fed microbial supplementation to potentially alleviate the anti-nutritional components of soy products, possibly improving bird performance, nutrient availability, as well as immune and intestinal health. A primary driver behind this research is the increase in cost of soy products in poultry rations due to increasing demand and decreased availability.

Need for nonnutritive feed additives

Least cost formulation of livestock rations is crucial in minimizing production costs and maximizing profit margins. Feed accounts for approximately 70% of the total cost of pig and poultry production (Barletta, 2010). With the rising costs of dietary energy sources, mainly soybeans, corn, and oil, alternative means of deriving energy from feed ingredients are being investigated. Enzymes have potential to increase overall feeding value, potentially improving or maintaining poultry performance while allowing the use of lower cost ingredients. Pectinase, cellulase, β-mannanase, xylanase, α-amylase, and β-glucanase are among enzymes that are often utilized in poultry diets containing soybean meal to potentially alleviate the anti-nutritional NSP and to increase nutrient utilization in corn-soybean meal based diets (Bach Knudsen 1997; Kocher et al., 2003).

Researchers examined broiler performance from 1 to 21 d with soybean meal or a soybean meal sunflower combination as the primary protein source. Replacement of approximately 25% soybean meal with sunflower meal significantly improved growth rate, feed intake, and feed conversion. When an enzyme cocktail containing pectinase, galactanase, arabinanase, β-glucanase, and polygalacturonase (380, 420, 140, 480, 530 units/g) were added to these same soybean meal or sunflower meal combination diets no improvements in performance were observed. Enzyme addition did increase the concentrations of raffinose and sucrose in the proximal small intestine. Overall, when birds were fed soybean meal as the primary protein source, an increase in the concentration of oligosaccharides present in the digesta from the distal small intestine demonstrated significant negative impacts on broiler performance (Irish and Balnave, 1993).

In vitro research utilizing a multi-enzyme preparation, isolated from *Aspergillus aculeatus*, determined type 1,4-β-arabinogalactanase led to effective solubilization of dry matter in soybean meal. Another enzyme, 1,4-β-galactomannanase, demonstrated release of sugar from untreated (lacking previous pepsin degradation) soybean meal. Synergistic impacts relating to use of galactanase and mannanase on endo-polygalacturonases were also noted (Vahjen et al., 2005). In vivo work was also conducted with galactanase originating form *H. insolens* solely and in combination with a mannanase enzyme. From 7 to 35 d broilers were fed corn and soybean meal (48% CP) rations supplemented with a combination of both enzymes. Compared to galactanase alone, additional supplementation of mannanase decreased live weight gain and feed intake. Researchers concluded that 1,4-β-arabinogalactanase enzymes are promising for use in soybean meal based diets, and combination with endo-polygalacturonases should also be considered. In this experiment partial hydrolysis of NSP did result in enhanced digestibility; however, increased hydrolysis, with increased NSP degrading enzyme (mannanase) addition, may produce oligo- and monomers with anti-nutritive impacts (Vahjen et al., 2005).

Exogenous enzyme products increase availability of nutrients already present in feedstuffs (Zanella et al., 1999; Kocher et al., 2003; Rutherfurd, 2007). Poultry have limited endogenous secretion of NSP-degrading enzymes, reducing utilization of these nutrients in the small intestine (Angkanaporn et al., 1994). Exogenous enzyme addition has been shown effective in improving ileal digestible energy of soybean meal (Douglas et al.,2000). Apparent ileal digestibility of CP was increased by 2.9% when male Hubbard broilers were fed an exogenous enzyme cocktail (0.1% inclusion of 800 µ/g xylanase, 6,000 µ/g protease, and 2,000 µ/g amylase) from 1 to 45 d (Zanella et al., 1999). Apparent ileal digestibility of

nitrogen was also observed to increase by 6.6% when 0.5% exogenous enzyme (α-amylase, β-glucanase, and xylanase) was fed from 21 to 28 d to male Ross 308 broiler chickens (Rutherfurd et al., 2007). Nutrients are often bound by cell walls rich in NSP limiting endogenous digestive enzyme access to these nutrients, thereby decreasing digestibility. The advantageous effects of exogenous enzymes on complementing and increasing endogenous enzyme activity are beneficial for improving overall feeding value of soy products (Bedford, 1996).

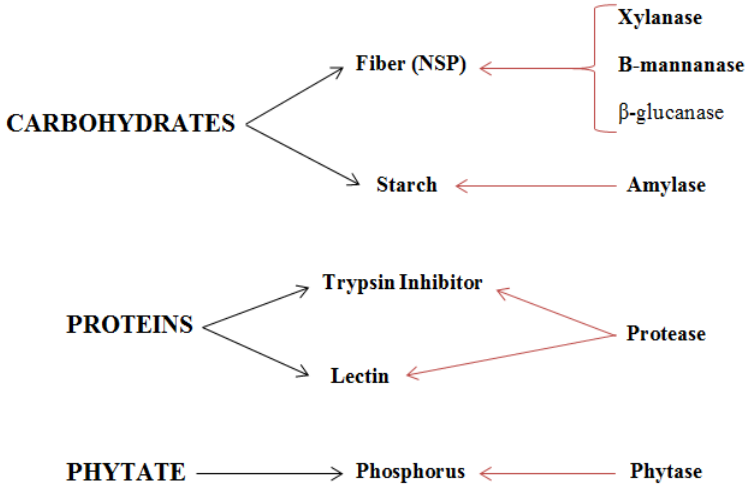

Figure 2.5. Substrates and anti-nutrients targeted by some commonly utilized exogenous enzymes. Adapted from Bedford and Partridge (2010).

Exogenous xylanase amylase protease enzyme

Exogenous supplementation of xylanase, amylase, and protease enzymes (Figure 2.5) are used to target NSP, starch, and protein consequently improving digestion in monogastric animals fed corn-soybean meal diets (Burnett, 1996). Xylanase is a general descriptor for several classes of enzymes that have varying modes of action but all result in the hydrolysis of the xylan component of NSP present in dietary ingredients (Paloheimo et al., 2010). Hydrolysis of soluble NSP, mainly in wheat based diets, results in decreased digestive

viscosity leading to increased digesta passage rate, which decreases bacterial proliferation in the small intestine, endogenous secretions, and enzyme production. Collectively, this results in increased nutrient sparing and digestibility (Brenes et al., 1993; Jozefiak et al., 2007; Pirgozliev et al., 2010). Hydrolysis of both soluble and insoluble NSP decreases water holding capacity of the gastrointestinal tract resulting in increased digesta flow and nutrient diffusion, and hydrolysis of insoluble NSP can also increase cell wall permeability leading to increased nutrient diffusion and digestibility (Bedford 1996; Zanella et al., 2004).

Breakdown of plant cell wall components allows increased access by pancreatic amylases and proteases which are capable of releasing starch and protein. Release of starch and protein can result in increased animal performance through increased growth and feed utilization along with decreased gastrointestinal tract maintenance (Choct and Cadogan, 2001). Exogenous protease and amylase enzyme products are often supplemented as a cocktail with xylanase and have been shown to increase utilization of protein, in the upper gastrointestinal tract of broiler chickens, as well as digestion of starch encapsulated in endosperm (Zanella et al., 1999).

The addition of XAP enzymes were evaluated in corn-soybean meal diets using digestibility assays with 15 month old White Leghorn roosters for a 37 d period (Zanella et al., 1999). In this experiment birds were fed corn and soy (soybean meal, extruded full fat soybeans, or roasted full-fat soybeans) and supplemented with exogenous xylanase amylase protease (XAP) enzyme product (containing 800 μ/g xylanase, 2,000 μ/g amylase, and 6,000 μ/g protease) included at 0.1% of the diet. Ileal contents were sampled and the addition of XAP improved overall CP digestibility by 2.9% in comparison to the control diet, along with specific improvements in valine and threonine digestibility of 2.3 and 3.0%, respectively.

The enzyme complex also improved ileal starch digestibility by 1.8% and ileal fat digestibility by 1.6%. These same corn and soy diets supplemented with XAP were fed in a performance based trials to day old Hubbard male broilers for a 45 d period. Enzyme supplementation reduced feed conversion ratio by 2.2% and improved body weight by 1.9%. Silversides and Bedford (1999) hypothesized that supplementation of exogenous multi-enzyme cocktails in corn-soybean meal diets may increase starch utilization in the proximal small intestine, by increasing the time endogenous secretions and the microbial flora have to act on nutrients.

A commercial XAP enzyme (300 U xylanase/g diet, 400 U amylase/g diet, and 4,000 U protease/g diet) was utilized in a series of experiments at 0.10% inclusion. Diets containing high NSP wheat cereal grain were fed to turkey hens from 3 to 9 wk of age. Addition of XAP resulted in significant improvement in feed conversion ratio and AMEn, over diets without enzyme supplementation. In addition to the increased AMEn, starch disappearance from the proximal small intestine was also increased in these hens (Persia et al., 2002). Another experiment utilizing XAP enzyme (800 U/g xylanase, 2,000 U/g amylase, and 6,000 U/g protease) showed significant improvement in ileal digestible energy of the corn-soybean meal diet when fed to commercial male broilers from 8 to 21 d. No significant effect of enzyme supplementation on bird weight gain was observed and impact of XAP enzyme supplementation varied depending on soybean meal source in this experiment (Douglas et al., 2000).

Although XAP is a common combination of enzymes for use in commercial poultry production, other combinations have been evaluated. Marsman and others (1997) evaluated the effects of protease and carbohydrase combination, on growth performance and ileal

nutrient digestibility of broiler chickens. Both enzymes were supplemented alone and in combination to diets containing soybean meal as the primary protein source. Day old female Ross broilers were fed a control diet with casein as the main protein source from 1 to 7 d. From 7 to 25 d birds were transitioned to experimental diets. Performance parameters, body weight gain, feed intake, and feed conversion were not affected by enzyme treatment. Carbohydrase enzyme treatment significantly increased ileal NSP degradability and overall enzyme treatment improved apparent ileal crude protein digestibility over the treatment groups with no enzyme. Increases observed in CP and NSP degradation may result in improved body weight gain and overall performance parameters if enzyme was supplemented from day of hatch.

The influence of supplementation of exogenous xylanase (200 mg/kg diet), derived from *Thermomyces lanuginosus,* and protease (200 mg/kg diet) from the fermentation of *Bacillus licheniformis* and transcribed genes from *Nocardiopsis prasina* on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal based diet was examined by Kalmendal and Tauson (2012). Day old straight run Ross 308 broiler chicks were fed for a 34 d period. Results show enzyme supplementation had no significant effect on body weight gain and feed intake of birds at any time point. Xylanase and protease supplementation, alone or the combination, significantly improved feed conversion ratio (1.43 g/g) and feed AMEn (3,389 kcal/kg) compared to the control FCR and AMEn of 1.47 g/g and 3,271 kcal/kg respectively. Apparent digestibility of starch and fat was significantly improved with all enzyme treatments; however, apparent retention of CP was not affected.

Another experiment utilized the same specific xylanase (derived from *Thermomyces lanuginosus*) provided at 100 g/kg diet, and showed significantly increased body weight gain in Ross 208 broilers fed wheat based diets from 15 to 42 d (Engberg et al., 2004). Increased wheat ingredients in the second experiment combined with xylanase may have resulted in increased hydrolysis of soluble NSP and reduced digesta viscosity (Kocher et al., 2002) leading to increased nutrient diffusion and energy availability for growth (Bedford, 1996).

Breakdown of plant cell wall carbohydrates by NSP enzymes eventually leads to production of short-chain oligosaccharides (di- and tri-saccharides). Oligosaccharides provide substrate for bacterial fermentation, and can also be beneficial in volatile fatty acid production (Choct and Cadogan, 2001; Paloheimo et al., 2010). When NSP degrading enzymes have been used in higher concentrations, work suggests that oligosaccharides can be further degraded to monosaccharides (Fuller, 2001; Choct. et al., 2010). Excess monosaccharides in the digestive tract have the potential to cause osmotic diarrhea as well as poor performance (Schutte, 1990). The effects of feeding graded amounts (25, 50, and 75 g/kg) of D-xylose or L-arabinose fed a diet based on corn, corn starch, and soybean isolate on performance was examined. All diets were formulated to be isocaloric and were provided to broiler chickens from 6 to 27 d of age. A significant negative effect of both pentose sugars (xylose and arabinose) was reported for body weight gain. Birds fed D-xylose diets had significantly decreased feed intake with increasing pentose sugar concentration. Water intake increased linearly as the dietary concentrations of both pentose sugars was increased, and consequently dry matter content of the droppings decreased.

Supplementation of exogenous XAP targets a number of substrates and complements endogenous enzymes potentially leading to increased nutrient digestibility and metabolic

efficiency. Variation in bird performance and nutrient digestibility response has been observed when feeding soy ingredients and supplementing with multi-enzyme cocktails. Hydrolysis of NSP may lead to production of oligosaccharides that have potential to positively modulate the gastrointestinal tract and immune function. However an over-abundance of short-chain oligosaccharides or pentose sugars in the gastrointestinal tract may lead to digestive inefficiency. All potential impacts on digestibility and efficiency, both positive and negative, should be considered when supplementing multi-enzyme combinations to broiler chickens.

Exogenous β-mannanase

Identifying and alleviating anti-nutritional factors that reduce or inhibit nutrient utilization is a necessity for successful poultry production. Among these anti-nutrients are NSP or fiber which includes β-mannans (Daskiran et al., 2004). Beta-mannanase hydrolyzes β-mannan and is the main activity of the fermentation product of *Bacillus lentus*. Two of the main β-mannans in soybean meal are β-galactomannan and β-glucomannan (Zou et al., 2006). It is thought that β-mannanase has three primary modes of action against β-mannans, these mechanisms include decreased viscosity, improved energy metabolism, and reduced innate immune stimulation (Jackson et al., 2004).

Evaluation of β-mannanase on corn-soybean meal based diets with variable β-mannan content has been conducted using guar gum to alter β-mannan levels as its chemical structure is similar to that of β-mannan in soy (Daskiran et al., 2004). Experiment one focused on evaluating the effects of varying β-mannan concentration (0, 0.5, 1, and 2%) on performance, MEn, and net energy for gain of day old Cobb x Cobb male broilers supplemented with a β-mannanase. Experimental diets were fed for 2 wk and β-mannanase enzyme activities were

determined to be 152.7, 127.7, 145.9, and 141.5 million units per ton for the four treatment groups. Data show enzyme supplementation improved feed efficiency, ME_n, and net energy gain at all β-mannan inclusions, relative to the control group. As expected, increasing guar gum (β-mannan) inclusion decreased bird weight gain especially at the 2% inclusion as weight gain was only 153.8 g in comparison to the 178.6 g of the control fed birds. Similar broiler performance results have been reported with 2% guar gum levels in previous research (Vohra and Kratzer, 1964; Ray et al., 1982).

A second set of experiments, utilizing the same broiler stock type (Cobb x Cobb) evaluated the effect of incrementally increasing β-mannanase supplementation (0, 0.05, 0.1, 0.15%) with activities of 1.4, 145.9, 225.8, 349.8 million units/ ton respectively. Enzymes were added to a corn-soybean meal based diet with 1% guar gum inclusion from 1 to 14 d. Increasing enzyme supplementation did not influence final body weight, but did significantly improve feed:gain ratio at all inclusions (1.533 g/g compared to 1.495, 1.493, and 1.454 g/g). As in the first set of experiments MEn improved with increasing β-mannanase supplementation (control at 2,971 kcal/kg compared to 0.15% inclusion at 3,061 kcal/kg) (Daskiran et al., 2004). As seen with previous exogenous enzyme supplementation research, increasing duration of enzyme inclusion could produce differences in body weight gain especially due to significant differences in MEn values compared to control and supplemented diets.

Degradation of β-mannans can produce mannan oligosaccharide (MOS) which has been shown to positively influence the immune system in poultry. Zou and others (2006) hypothesized that the inclusion of β-mannanase in corn-soybean meal based broiler diets would improve growth performance and immunity. Results showed inclusion of exogenous

β-mannanase (0, 0.025, 0.05, and 0.075%) increased weight gain and feed conversion ratio in birds during the 4 to 6 wk and 0 to 6 wk periods. All inclusions of β-mannanase resulted in improvement in weight gain compared to the non-supplemented control. Feed conversion ratio was significantly improved (1.637 g/g) at 0.05% inclusion compared to all other treatments from 1.684 to 1.720 g/g. Performance improvements may have been observed due to the extended experimental trial duration compared to previously described research. Serum IgA, IgG, and IgM concentrations were also measured to determine the impact of supplementation on immune factors. A significant increase of 0.286% IgM was noted for 0.025% inclusion during the 3 to 6 wk period; however, no difference in IgG and IgA concentrations occurred during the trial period. T lymphocyte production was significantly improved in 6 wk old broilers at 0.05% β-mannanase inclusion (Zou et al. 2006). Results from this experiment demonstrate the potential beneficial impacts, on performance parameters and immune function, of including β-mannanase in broiler diets containing corn and soybean meal.

Another manuscript reported supplementing exogenous β-mannanase and pectinase enzymes targeted the breakdown of soy cell wall components of the diet, to a greater extent compared to corn (Jackson et al., 2004). Four experimental treatments were utilized supplying 0, 50, 80, and 110 MU (1 MU=10^6 enzyme activity units) of β-mannanase to broilers fed corn and soybean meal based rations. Feed intake was significantly increased in diets containing 80 MU/ton compared to 50 MU/ton β-mannanase. Despite the reduced feed intake, both 80 MU/ton and 110 MU/ton inclusions resulted in significantly increased weight gain and feed efficiency in comparison to 50 MU/ton. Regression analysis indicated, for every 100 MU/ton β-mannanase supplemented, weight gain was improved by 120 g and feed

conversion ratio improved by 0.067 g of feed/g of gain. Clearly β-mannanase supplementation has the ability to impact broiler performance; however, concentration of β-mannanase supplemented is important in understanding the bird response.

Hydrolysis of mannans and galactans by commercial galactanase and mannanase exogenous enzymes in diets containing corn and soybean meal have resulted in improved broiler performance when fed from 1 to 28 d (Centeno et al., 2006). Day old Ross 308 broilers were supplemented with three separate exogenous enzyme preparations designed to target arabinoxlyans, β-1,3-1,4-glucans, and raffinose an oligosaccharides. Supplemented diets resulted in shortened ceca. Dietary fiber can cause an increase in muscle development of the intestine in response to indigestible nutrients and microbial activity. Therefore shortening of the ceca may indicate improved intestinal hydrolysis of mannans and galactans decreasing the amount of fermentation occurring in the caecum.

Utilization of β-mannanase supplements in broiler rations has the potential to positively influence bird performance, nutrient utilization, and immune function. These effects could lead to an increase in broiler efficiency, decreased endogenous secretions, and reduced cecal fermentation. When β-mannanase is supplemented into broiler rations containing mainly corn and soybean meal at hatch it has the ability to increase productivity and therefore profitability of the bird for the remainder of its production cycle. However research regarding the effects of increased soy ingredient inclusion along with combinations of β-mannanase and other exogenous enzymes is novel.

Direct-fed microbial

Probiotics are marketed as direct-fed microbial (DFM), and can be utilized as alternatives to therapeutic antibiotic supplementation. The use of DFM can reduce incidence

of enteric disease challenge and improve health status of an animal by maintaining a beneficial microbial population (Fuller, 2001; Lee et al., 2010). Some commonly used DFM include strains of yeasts *Lactobacillus spp.*, *Bacillus spp.*, *Streptococcus spp.*, and fungi *Aspergillus spp*. (Fuller, 2001). These DFM benefit the bird through regulation of microbial homeostasis in the intestine as well as maintenance of barrier function of the gastrointestinal system (Salminen et al., 1996). Supplementation of DFM can also interfere with the ability of a pathogen to colonize the gastrointestinal tract and infect mucosa (Gill, 2003). These and other gut health benefits stem from the process of competitive exclusion of pathogenic bacteria (Nurmi and Rantala, 1973; Schneitz, 2005), potentially resulting in improved bird performance (Corrier et al., 1995) and immune function (Schneitz, 2005).

Impacts of DFM on broiler performance, gut morphometry, and immune parameters have been well researched. Maintenance of the mucosa lining the gastrointestinal tract is important in preserving barrier function and animal immunity (Denbow, 2000; Gill, 2003). Day old broiler chicks were given access to non-supplemented mash diets from 1 to 2 d and supplemented with DFM from 3 to 22 d (Lee et al., 2010). Eight differing strains of *B. subtilis* and one multi-strain DFM were fed (1.5×10^5 cfu/g DFM) and data showed no significant alteration in body weight gain; however, intestinal morphometry was altered by supplementation. Significant increases in villus height and reduced serum levels of α-1-acid glycoprotein, an indicator of inflammation, were observed in DFM treated broilers, compared to non-supplemented controls. Increased villus height is associated with increased absorptive capacity of nutrients (Denbow, 2000) and it has also been demonstrated that DFM are capable of increasing nutrient absorption in the human gastrointestinal system (Hooper et al., 2002).

Direct-fed microbials may also be involved in regulation of hind gut fermentation and synthesis of short-chain fatty acids and can increase the efficiency of gut microbes to ferment NSP (Sakata, 1987). Short-chain fatty acids are believed to enhance sodium absorption, stimulate blood flow and regulate nutrient absorption. Increases in cecal SCFA concentrations, especially increases in butyrate concentration have been documented. These SCFA can play an important role in meeting the energy requirements of the bird (Caballero-Franco et al., 2007). Not only do these SCFA provide the bird with a substitute for energy production, butyrate is also effective in promoting proliferation and functional maturation of intestinal epithelial cells. These consequences result in increased arterial blood flow which is linked to nutrient absorption (Cherbut, 2003).

Male broiler chickens were fed corn-soybean meal based diets supplemented with a commercial probiotic (0, 0.2, 0.4, or 0.6%) and antibiotic (0 or 6 ppm flavomycin) from 1 to 42 d (Li et al., 2008). Starter diets were fed from 1 to 21 d and birds were then transitioned to grower diets for d 22 to 42. Supplementation with DFM did not result in significant increase in body weight gain or feed intake during the 42 d period. However DFM inclusion consistently improved the apparent ileal digestibility of CP and amino acids. Probiotic supplementation also significantly improved ileal digestibility of dry matter, energy, calcium, and phosphate. From these data, DFM could be an effective alternative to antibiotic (flavomycin) use in broiler chicken production.

Others have found supplementation of DFM in broiler rations has improved performance, feed efficiency, and body weight gain, for broilers fed corn-soybean meal diets (Jin et al., 1998) and for broiler chicks fed DFM for 45 d (Awad et al., 2009). Additionally supplementation with DFM in corn-SBM diet fed to laying hens has resulted in reduced feed

intake, and improved feed conversion ratio (Balevi et al., 2001; Nahashon et al., 1994). Overall improvements in intestinal health and nutrient digestibility are important implications of DFM use in poultry production. Improvements in bird performance parameters such as feed intake and feed conversion ratio have also been reported and might result from increased energy availability due to increased nutrient digestibility.

Conclusions

Presence of anti-nutritional factors in soy products presents a challenge when fed in increased concentrations to poultry. Some anti-nutrient properties can be eliminated through further processing methods such as heat treatment, while others such as NSP, oligosaccharides, and phytates are targeted through supplementation of exogenous enzymes. Commonly utilized carbohydrate targeting enzymes include β-mannanases and xylanases. Results of supplementation of these exogenous enzyme combinations on bird performance parameters have been mixed. Overall, positive impacts on bird body weight gain, feed efficiency, and nutrient digestibility have been noted when multi-enzyme additives are used alone in corn-soybean meal based diets. Supplementation of DFM constructively modulates microbial flora and barrier integrity while reducing pathogen colonization and increasing SCFA production. It is clear that precise enzyme concentration and dietary substrate considerations are critical in observing desired animal response.

Expected results when utilizing a combination of a multi-enzyme, such as XAP and β-mannanases include increased nutrient digestibility. β-mannanases target mannan sugars whereas xylanases primarily target xylans or arabinoxylans present as NSP. Since β-mannanases and xylanases target two differing substrates they could work in an additive

manner. As the xylanase and β-mannanase break down carbohydrates in the cell walls of soy ingredients, release of proteins and starches into the gastrointestinal tract occurs. Amylase and protease enzymes are then able to assist in digestion of these substrates therefore increasing the nutritive value the bird is able to get from the diet. Increased bird growth performance, nutrient digestibility, and feed efficiency may result.

When the combination of XAP and DFM are utilized similar results might be expected. As stated previously, the enzyme cocktail contained in a XAP supplement could result in breakdown of cell wall materials triggering the release of nutrients for enzymatic degradation and eventual intestinal absorption. Utilizing a DFM increases the beneficial microbial population in the gastrointestinal tract of the bird and competitively excludes pathogenic organisms, leading to improved immune function. Direct-fed microbials could utilize oligosaccharides and polysaccharide substrates to produce SCFA, which could also provide a viable energy source for the bird. Overall, improved immune function should decrease energy required for maintenance and XAP and DFM action should also increase energy available for metabolism. Therefore supplementation with a combination of XAP and DFM should increase bird growth performance, nutrient digestibility, and feed efficiency. Addition of β-mannanase into the combination of XAP and DFM should provide further benefit to the bird.

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CHAPTER 3. EFFECTS OF XYLANASE AMYLASE PROTEASE AND BETA-MANNANASE ENZYMES IN COMBINATION WITH VARIABLE SOY INCLUSION LEVELS ON BROILER PERFORMANCE AND METABOLIZABLE ENERGY

Abstract

Soy products are widely utilized in poultry rations due to high protein content and relatively well-balanced amino acid profile. The objective of this experiment was to determine the effects of enzyme combinations on broiler chicken performance and metabolizable energy, when fed diets containing various concentrations of soy products. Twelve experimental diets were arranged as a 3 x 4 factorial. Three different inclusions of soy products, low (20%), intermediate (28%), and high (35%) were fed with four combinations of xylanase amylase protease (XAP) and β-mannanase (MAN) enzymes. Experimental diets were fed from 10 to 21 d. Nitrogen corrected apparent metabolizable energy, body weight gain (BWG), feed intake, feed efficiency (FE), and feed conversion ratios (FCR) were calculated. Data were analyzed using the two-way ANOVA procedure of SAS. Fisher's LSD was used to separate significant least squares means with the probability set at $P \leq 0.05$. Fisher LSD was performed only on the results with an ANOVA (overall $P \leq 0.05$). There were no significant differences in BWG, low soy inclusion resulted in increased ($P \leq 0.01$) broiler feed intake. Broiler feed intake decreased ($P \leq 0.01$) with XAP and β-mannanase treatments compared to the non-enzyme supplemented diets. Low soy un-supplemented diets resulted in reduced FE ($P = 0.02$) and increased FCR ($P = 0.01$). Enzyme supplementation had no effect on AMEn in either the low or intermediate soy diets, but XAP and β-mannanase reduced AMEn ($P = 0.05$) in comparison to the XAP and half dose of β-mannanase in high soy treatments. Overall, low soy treatments increased feed intake and

tended to decrease body weight gain potentially due to reduced nutrient availability compared to higher soy inclusions. All enzyme supplementation improved FE and FCR and AMEn was increased in high soy diets with XAP+½MAN compared to high soy diets with XAP+MAN. Reduction in AMEn with additional MAN inclusion may be due to energy depressing effects of carbohydrase enzymes at increased concentrations.

Key words: soy, multi-enzyme, β-mannanase, broiler, performance

Introduction

Soy is a fundamental component in commercial poultry diets and is valued for its high-quality protein meal and oil (Centeno et al., 2006). Soybean oil is prominently utilized for human food as well as industrial uses and is favored over other vegetable oils due to a high level of unsaturation along with other ideal chemical and physical properties (Perkins, 1995). However many anti-nutritional factors exist in soybean meal and other soy products used in broiler rations. Some of these anti-nutrients include oligosaccharides (raffinose and stachiose), phytates, trypsin inhibitor, non-starch polysaccharides, and β-mannans (Marsman et al., 1997; Kocher et al., 2003).

Anti-nutritional components, like non-starch polysaccharides **(NSP)**, found in feed ingredients are not able to be digested by endogenous enzymes and can inhibit normal digestion leading to digestive distress and poor animal performance (Vahouny et al., 1981). Nutrients such as starches, proteins, and minerals which are enclosed in fiber containing cell walls or chemically bound are not accessible to the animal and therefore cannot contribute to production (Choct and Annison, 1990). Also, developing gastrointestinal tracts of immature

animals, such as chicks, do not possess the capability to produce all important endogenous enzymes and cannot fully utilize feedstuffs. Exogenous enzymes can be utilized in animal feeds to address these issues and improve the nutritive value of feedstuffs (Barletta, 2010).

Substrates targeted by xylanase amylase protease **(XAP)** enzymes are primarily fiber, starch, and protein. Supplementation of exogenous XAP in diets containing corn and soy protein sources has been shown to increase ileal crude protein digestibility along with digestibility of amino acids such as tyrosine, cysteine, and valine (Zanella et al., 1999). Soybean meal contains approximately 6% soluble NSP and approximately 18 to 20% insoluble NSP (Bach Knudsen, 1997). Xylanase enzymes target NSP, resulting in plant cell wall destruction and therefore nutrient release (Choct and Cadogan, 2001; Paloheimo et al., 2010). Breakdown of plant cell wall carbohydrates leads to production of short-chain oligosaccharides (di- and tri-saccharides) creating substrate for bacterial fermentation, favorably altering bacterial populations within the gut (Schneeman et al., 1985; Thammarutwasik et al., 2009). With increasing concentration of soy products, soluble NSP form viscous gels that trap nutrients and slows passage rate of digesta (Fransen, 1999). Fiber can also hold water and trap water-soluble nutrients and create bulk in the gut slowing passage rate while reducing feed intake and subsequently growth (Paloheimo et al., 2010).

An experiment utilizing a commercial XAP in corn-soybean meal diets showed significant improvement in ileal digestible energy when supplemented to rations fed to broiler chickens from 8 to 21 d however no increase in growth performance was noted (Douglas et al., 2000). The effect of supplementation of exogenous xylanase **(XYL)** and protease **(PRO)** enzymes on performance, nutrient utilization, and intestinal morphology of 1 to 34 d old broiler chickens fed a wheat-soybean meal based diet was investigated. There was

no effect on body weight gain **(BWG)** and feed intake of birds at any time point. However XYL, PRO, and XYL+PRO supplementation significantly improved feed conversion ratio **(FCR)** and feed AMEn throughout the entire trial (Kalmendal and Tauson, 2012). Overall XAP and other enzyme combinations show potential to positively impact bird performance and nutrient utilization (Zanella et al., 1999; Cowieson and Adeola, 2005).

β-mannanase **(MAN)** is an enzyme that hydrolyzes β-mannan (Zou et al., 2006; Kocher et al., 2003). Soybean meal 48, or dehulled soybean meal, contains NSP in the form of pectic polysaccharides, 1,4-β-arabinogalactans and 1,2-1,4-β-rhamnogalacturonans (Vahjen et al., 2005). Three accepted modes of action for MAN include decreasing viscosity, improving energy metabolism, and reducing innate immune stimulation (Jackson et al., 2004). Research utilizing MAN, in corn-soybean meal broiler diets, show significant improvements in BWG and FCR. Two 14 d experiments were conducted utilizing guar gum to alter β-mannan levels, due to its chemical structure similarities to β-mannan in soy. Results of experiment 1 showed MAN enzyme supplementation improved feed efficiency **(FE)**, AMEn, and net energy gain. However in experiment 2 final BW was not improved but feed:gain ratio was improved at all MAN inclusions (Daskiran et al., 2004). β-mannanase products have been shown to positively impact broiler performance, uniformity, and immune status of broiler chickens.

Although the implications of feeding enzymes solely and some cocktails are well researched, other combinations are relatively unexplored. Another novel area of exploration is the performance and nutrient digestibility impacts of feeding variable soy concentrations to broiler chickens. The objective of the current experiment was to determine the effects of

XAP and MAN inclusion on broiler growth performance and nutrient utilization when rations with various concentrations of dietary soy were fed from 10 to 21 d.

Materials and Methods

Experimental Design

All animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University before the start of the experiment. A total of 1,152 day-old Ross 708 male broiler chicks were obtained from a commercial hatchery (Welp Hatchery, Bancroft, IA) and transported to the Iowa State University Poultry Research and Teaching Unit (Ames, IA.). All birds were fed a corn-soybean meal based starter diet, with addition of toasted full fat soybeans, from 0 to 9 d. On d 10 all birds were sorted into groups by weight, wind-banded, individually weighed, assigned to cages to minimize differences in initial weight, and transitioned to experimental diets. The experiment utilized a 3 x 4 factorial arrangement of treatments with 3 soy inclusion levels [20% (low), 28% (intermediate), and 35% (high)] and 4 enzyme treatments [none, 100 g/MT xylanase amylase protease (XAP), 100 g/MT XAP with 100 g/MT β-mannanse (XAP + MAN), and 100 g/MT XAP with 50 g/MT β-mannanse (XAP + ½MAN)]. Birds were allocated to 12 experimental units **(EU)** for each of the 12 treatments, with an EU defined as 8 birds caged together. To produce desired replication, the experiment was conducted over two time periods. The first time point included 8 replicates from each treatment while the second time point included 4 replicates from each treatment, creating a total of 12 replicates per treatment.

Birds were provided *ad-libitum* access to feed and water throughout the experiment. Two experimental diets were formulated to have high soy and low soy concentrations. High

soy content was produced using the combination of toasted full fat soybeans and soybean meal 48. Low soy contained reduced soy protein which was replaced with dried distillers grains with solubles, canola meal, meat and bone meal, and synthetic lysine. From these two diets, three concentrations of dietary soy inclusion were generated including low (20% soy products), intermediate (28% soy products), and high (35% soy products) by using either diet or a 50/50 mixture of the two diets (Table 3.1). Titanium dioxide was added to all experimental diets at a rate of 0.5% as an inert dietary marker to determine AMEn (Scott et al., 1982). All diets were manufactured at Iowa State University Poultry Research and Teaching Unit and were fed in mash form.

Birds were housed in Petersime battery cages (65.8 $in.^2$/bird) with continuous light. Room temperature was maintained at 85^0F at the start of chick housing. Temperatures were reduced 5^0F each week until reaching a final temperature of 70^0F at the end of the experiment. Supplemental heat, via battery cage heating units, was available to all chicks allowing birds to determine optimal thermal climate.

Data Collection

Birds were monitored twice daily with mortality removed from pens as they occurred. Mortality or cull birds were weighed and recorded before disposal. Feed intake was determined for each cage by calculating the difference between the amount of feed offered and the amount of feed refused (initial feeder weight and feed + feed added – final feeder weight and remaining feed) and reported for the 10 to 21 d period. Individual bird weights were recorded at 10 and 21 d with BWG reported for the 10 to 21 d period. Chick FCR and feed efficiency **(FE)** were determined for the 10 to 21 d period from BWG and feed intake

data. Feed efficiency is a ratio of grams of gain to kilograms of feed consumed, and FCR is a ratio of grams of feed consumed to grams of gain. Both ratios were calculated on a pen basis and were corrected for mortality by adding mortality weight gain to the total pen weight prior to calculation. Pans below each EU were cleared of excreta, feathers, and feed at 19 d and 3 d of fresh and clean excreta samples were collected from pans below each cage on d 21 for AMEn determination. All samples were immediately transported to the laboratory and frozen until analysis. On d 21 all birds were euthanized via CO_2 to collect ileal contents and leg samples. The ileum was defined as the segment from the point of Meckel's diverticulum to the ileo-cecal junction. Contents of the ileum were gently expressed with all ileums from each EU combined to generate one sample. Legs were sampled from three randomly selected birds per EU for tibia (Persia et. al., 2006) and toe ash protocol modified from Persia et. al. (2006) as well as bone breaking strength determination (Powell et al., 2008). Three tibia bones from each EU were analyzed using a TA.XTplus Texture Analyser. (Texture Technologies Corporation, Scarsdale, New York). The ileum section was defined as the section between the Meckel's diverticulum and ileo-cecal junction. Ileal contents from individual birds were grouped by EU to generate a single sample and were later analyzed for ileal calcium and phosphorus digestibility (AOAC International, 1999). Data from ileal and leg sample collection will not be discussed in this thesis work.

Laboratory Analysis

Excreta and ileal samples were oven dried for 3 days at 65^0C (Jacobs et al., 2011). Feed samples were also corrected to a dry matter basis by measuring 0.5g of each sample and drying them in an oven at 100ºC for 24h (Yamato Scientific America Inc., Santa Clara, CA).

After drying, excreta and ileal samples were ground through a 1.0 mm screen (Whiley) and feed samples were ground through a 0.5-mm screen (Brinkmann Instruments Inc., Westbury, NY). Feed samples were sent to Experiment Station Chemical Laboratories (University of Missouri, Columbia, MO) for proximate analysis. Ileal samples were sent to Central Analytical Laboratory (University of Arkansas, Fayetteville, AR) to determine calcium and phosphorus digestibility using the method published in "Analytical techniques for inorganic contaminants" (AOAC International, 1999). Titanium dioxide was determined (Leone, 1973) for feed and excreta samples. Gross energy (GE), for excreta and feed samples, was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., IL). Nitrogen content of excreta and feed samples was determined using LECO Trumac N (LECO Corp. St. Joseph, MI.). Nitrogen corrected apparent metabolizable energy was calculated with GE, nitrogen content, and titanium dioxide concentration using the equation by Scott *et al.* (1982), chromic oxide marker was replaced with titanium dioxide. Dietary AMEn values for each diet were calculated as follows:

AMEn= dietary GE - [excreta GE × dietary Ti/excreta Ti – 8.22 × (dietary N - excreta N × dietary Ti/excreta Ti)]

Three leg samples from each EU were autoclaved to remove tissue and cartilage caps. Tibias were then oven dried at 100^0C for 24 h and ashed in a furnace at 560^0C for 24 h to determine bone mineralization (Persia and Saylor, 2006). Middle toes, with tissue intact, from the three remaining leg samples of each EU were oven dried at 100^0C for 24 h and ashed in a furnace at 560^0C for 24 h to establish toe mineralization. The same three remaining leg samples for each EU, also utilized for toe mineralization, were utilized to determine bone breaking strength of bird tibias using a TA.XTplus Texture Analyzer with Texture Exponent Software (Texture Technologies Corporation, Scarsdale, New York) and a

procedure modified from Powell et al. (2008). Flesh remained intact for bone breaking strength analysis and a three point bend rig with a knife edge blade was used. Results of calcium and phosphorus digestibility, tibia ash, toe ash, and bone breaking strength will not be presented.

Statistical Analysis

Statistical analysis was carried out as a 3 x 4 factorial arrangement of treatments with a completely randomized design. Outliers were removed from data sets prior to analysis. The average of each treatment was calculated and two times the standard deviation of each treatment was added or subtracted from the calculated average. All values falling outside of the calculated range were eliminated. Two-way ANOVA procedure of SAS was used to analyze all treatments (SAS Inst. Inc., Cary, NC). Fisher's LSD was used to separate significant least squares means with the probability set at $P \leq 0.05$. Fisher LSD was performed only on the results with an ANOVA (overall $P \leq 0.05$). *P-values* up to 0.10 are discussed as tendencies.

Results

There were three mortalities reported and utilized to correct for FCR and FE values for the 10 to 21 d experimental period. Mortalities were not contained to one treatment and no treatment related patterns were noted. Body weight gain was not significantly altered by either soy inclusion or enzyme treatment, although low soy tended to reduce BWG ($P = 0.10$) when compared to intermediate and high soy inclusions (463, 479, 476 g/chick, respectively) (Table 3.2). As reported on a cage basis, low soy diets resulted in higher feed intake (5.65 kg/pen) then both intermediate and high soy inclusion diets, 5.48 and 5.39 kg/cage,

respectively ($P \leq 0.01$). Enzyme supplementation decreased feed intake compared to non-supplemented diets ($P \leq 0.01$). Significant interactions were noted in both FCR ($P = 0.01$) and FE ($P = 0.02$) as enzyme treatment of the low soy diet resulted in improved FCR and FE in comparison to the non-treated low soy diet. However there were no differences in FCR and FE regardless of enzyme treatment in both the intermediate and high soy inclusion diets. Another interaction was noted for AMEn as enzyme had little effect on AMEn in either the low or intermediate soy diets, but in high soy diets XAP and β-mannanase resulted in the lowest AMEn (3,060 kcal/kg) while the XAP and half dose of β-mannanase resulted in the highest AMEn (3,175 kcal/kg), although not significantly different from the control fed birds (3121 kcal/kg).

Discussion

Body weight gain was not significantly different in this experiment, although low soy diets tended to decrease bird BWG. Low soy diets had increased fiber concentrations from canola meal and dried distillers grain with solubles ingredients. Higher fiber diets are not usually efficiently utilized in the poultry gastrointestinal tract and may have resulted in reduced BWG in low soy treatments (Choct and Cadogan, 2001). Research examining protease and carbohydrase supplementation of rations containing primarily soybean meal protein observed no significant impact of enzyme treatment on broiler chicken body weight gain, from 7 to 25 d (Marsman et al., 1997). Similarly, when an enzyme cocktail containing β-glucanse, β-mannanase, and pectinase was utilized singularly or in combination with xylanase, amylase, and protease enzymes no significant effect on broiler chicken body weight gain was observed when birds were supplemented from 24 to 31 d of age (Kocher et al., 2003). When birds were supplemented from 8 to 21 d with an exogenous XAP, no

significant effect on bird weight gain was observed and the efficacy of enzyme utilization varied dependent on soybean meal source, due to differences in soy nutrient profile (Douglas et al., 2000).

Experiments utilizing mannanase products have observed decreased live weight gain and feed intake with mannanase enzyme supplementation (11,620 IU/kg) compared to non-supplemented controls (Vahjen et al., 2005). On the other hand, others have observed improvements in broiler growth performance with enzyme supplementation (Zanella et al., 1999; Engberg et al., 2004; Centeno et al., 2006). An experiment utilizing XAP in soybean meal based diets for a 1 to 45 d period resulted in significant impacts on broiler performance, with 1.9% improvement in body weight and 2.2% reduction in FCR (Zanella et al., 1999). Supplementation of exogenous XAP and mannanase enzymes appears to produce improvements in overall bird weight gain and efficiency when supplemented from day of hatch. Data from previous evaluations coincide with parameters measured during the 10 to 21 d experimental period and suggest supplementation should occur from day one.

In the current experiment, feed intake was reduced with XAP and MAN short term supplementation and also with increased soy inclusion. Broiler chickens reduced feed consumption when exogenous enzymes were added compared to the same diet without supplementation. Enzyme addition may allow birds to decrease feed consumption as a result of increased nutrient availability (Daskiran et al., 2004; O'Neill et al., 2012). Hydrolysis of insoluble NSP can increase cell wall permeability leading to increased nutrient diffusion and digestibility. Increased nutrient diffusion and digestibility result in increased animal performance through increased growth and feed utilization along with decreased gastrointestinal tract maintenance (Bach Knudsen, 1997).

Initially when formulating experimental diets high soy treatments were expected to result in decreased intake compared to all other inclusion levels due to the increase in anti-nutrient concentration. However, high soy inclusion did not limit intake as initially expected. Feed intake was increased in low soy diets possibly due to an increase in fiber content from DDGS and canola meal ingredients. Since fiber is not well utilized by the gastrointestinal tract of poultry feed consumption increases as a means to increase energy available from the diet. Increased soy inclusion resulted in decreased feed intake potentially due to increased protein quality associated with soybean meal (Asbridge, 1995) and nutrient availability as reflected by reported AMEn values. Additionally, there may have been limited anti-nutrients present in low soy diets that tend to reduce bird feed consumption (Marsman et al., 1997; Kocher et al., 2003).

All enzyme supplemented treatments, in the current experiment, resulted in improved FE and FCR compared to non-supplemented diets. Low soy without enzyme supplementation had the lowest FE and highest FCR of all treatments. As with BWG, an increase in fiber associated with low soy treatments from canola meal and DDGS ingredients may have contributed to increased FCR and decreased FE observed (Choct and Cadogan, 2001). Carbohydrase enzymes (xylanase, amylase, and β-mannanase) have been shown to improve BWG and FCR of broilers, when fed corn-soybean meal diets, due to increased NSP digestibility and ileal digestibility of crude protein, starch, and lipids.

Exogenous carbohydrase enzymes contain cell wall degrading activity, leading to increased nutrient digestibility and therefore increased efficiency (Marsman et al., 1997; Zanella et al., 1999). Several publications have reported improvements in FE and FCR relating to supplementation of exogenous enzyme products (Persia et al., 2002; Zou et al.,

2006; Kalmendal and Tauson, 2012). The improvements observed in FE and FCR with enzyme supplementation correlate with increased AMEn values for enzyme treated diets; however, FE and FCR for enzyme supplemented diets were not significantly different from control diets. This relationship between bird body weight gain, FE, and AMEn has also been documented in various publications (Daskiran et al., 2004; Kalmendal and Tauson, 2012). Ileal digestibility of protein and starch has also been shown to increase with carbohydrase addition, potentially contributing to improved AMEn with enzyme addition and therefore improved FE and FCR (Meng and Slominski, 2005).

Increased carbohydrase enzyme concentration could potentially lead to an energy depressing effect. Though the mechanism is not well understood, increased carbohydrase supplementation of corn and soybean meal diets could result in excessive endogenous secretion to address the nutrients released. Production of endogenous secretions such as enzymes and bile requires an energy source which could funnel energy away from growth. Excess nutrients could potentially flood the gastrointestinal tract resulting in epithelial destruction and increased fermentation in the lower tract.

Nitrogen corrected apparent metabolizable energy for high soy diets with XAP+½MAN compared to XAP+MAN. In another experiment, increasing levels of β-mannanase from 0.10% to 0.15% dietary inclusion significantly increased FCR and decreased BWG in broiler chicks (Daskiran et al., 2004). These results from Daskiran et al. (2004) could demonstrate an energy depressing effect of increasing carbohydrase concentrations. Other research examining the effects of enzyme products designed to degrade NSP, has shown no significant influence on AME, degradability of NSP, and FCR (Kocher, 2000). Although others have reported AMEn was significantly improved in diets containing

β-glucanse, β-mannanase, and pectinase utilized singularly or in combination with xylanase, amylase, and protease enzymes compared to non-supplemented diets (Kocher et al., 2003). One mechanism for increased AMEn proposed for enzymes containing β-glucanase, β-mannanase, and pectinase is solubilization of pectic NSP which release nutrients from cell walls of soybean meal products (Kocher**,** 2000). However there may be circumstances where supplementation exceeds the bird's ability to effectively utilize these nutrients resulting in overwhelming of the gastrointestinal tract and consequent energy depressing impacts (Daskiran et al., 2004).

The purpose of the current experiment was to provide a basis of understanding for supplementation of XAP and MAN exogenous enzyme products when fed in combination with variable soy inclusions to broiler on growth performance and nutrient digestibility. Broilers are able to perform well on high soy diets when fed at 35% inclusion, and fiber concentration of diet is an important consideration when evaluating the value of a broiler ration. Overall, inclusion of XAP and MAN could provide benefit when feeding diets containing primarily soy protein, however there seems to be a threshold where carbohydrase supplementation has an energy depressing impact on nutrient digestibility.

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Table 3.1. Composition of broiler starter and experimental diets fed from 0 to 9 d and 10 to 21 d, respectively. Soy concentrations for experimental diets were low (20%), intermediate (28%), and high (35%). xylanase, amylase, and protease enzyme cocktail and β-mannanase enzyme were added to generate final experimental diets.

Diet	Starter	Low Soy	Intermediate Soy	High Soy
Ingredient Composition	----------(%)----------			
Corn	51.83	51.85	51.18	50.52
Soybean meal (48% CP)	23.38	20.00	22.50	25.00
Soybeans (toasted full fat)	5.00	0.00	5.00	10.00
Dried distillers grains with solubles	10.00	15.00	12.48	9.95
Meat/bone meal	4.13	2.90	1.45	0.00
Canola meal	2.39	5.47	2.73	0.00
Soy oil	0.50	1.82	1.16	0.50
Salt	0.31	0.30	0.35	0.39
DL-methionine	0.29	0.20	0.21	0.22
L-threonine	0.05	0.00	0.01	0.02
Bio-Lys[1]	0.47	0.40	0.30	0.21
Limestone	0.92	0.84	0.97	1.11
Di-calcium phosphate	0.00	0.00	0.43	0.86
Choline chloride	0.10	0.10	0.10	0.10
Vitamin and mineral premix[3]	0.63	0.63	0.63	0.63
Phytase[2]	0.00125	0.00125	0.00125	0.00125
Titanium dioxide	0.00	0.50	0.50	0.50
Chemical Composition (Calculated)	----------(%)----------			
Crude protein	23.45	21.75	22.25	22.75
ME (kcal/kg)	2960	2960	2960	2960
Calcium	0.89	0.74	0.74	0.74
Non-phytate phosphorus	0.35	0.30	0.30	0.30
Total Fat	5.07	5.78	5.64	5.49
Total Fiber	3.38	3.68	3.49	3.31
Digestible Methionine	0.60	0.50	0.51	0.52
Digestible Lysine	1.27	1.10	1.13	1.15
Digestible Threonine	0.83	0.73	0.77	0.80
Digestible Methionine+Cysteine	0.94	0.84	0.85	0.86
Chemical Composition (Analyzed)	----------(%)----------			
Crude protein		22.53	22.82	23.39
Fat		5.49	5.03	4.48
Fiber		3.53	3.16	2.73
Methionine		0.53	0.52	0.53
Lysine		1.31	1.31	1.28
Threonine		0.81	0.84	0.86
Methionine+Cysteine		0.86	0.84	0.84

[1,2,3] Additional information provided in Appendix C.

Table 3.2. Effects of soy inclusion along with xylanase, amylase, and protease (XAP) and β-mannanase (MAN) enzyme on nitrogen corrected apparent metabolizable energy (AMEn), body weight gain (BWG), feed intake (FI), feed efficiency (FE), and feed conversion ratio (FCR) of 10 to 21 d old broilers.

Soy Inclusion	Enzyme	BWG	FI	FE	FCR	AMEn
		(g/ck)	(kg/pen)	(g/kg)	(g/g)	(kcal/kg)
Low		463	5.65[a]	676	1.48	3134
Intermediate		479	5.48[b]	690	1.45	3097
High		476	5.39[b]	686	1.46	3110
Pooled SEM		5.5	0.055	4.3	0.010	12.6
	None	468	5.71[a]	676	1.48	3121
	XAP	467	5.36[b]	681	1.47	3107
	XAP + MAN	476	5.46[b]	691	1.45	3104
	XAP + ½MAN	479	5.50[b]	689	1.45	3122
Pooled SEM		6.4	0.063	4.9	0.011	14.6
Low	None	470	5.97	645[b]	1.56[a]	3145[ab]
	XAP	450	5.49	685[a]	1.46[b]	3128[abcd]
	XAP + MAN	455	5.57	688[a]	1.46[b]	3138[abc]
	XAP + ½MAN	477	5.56	686[a]	1.46[b]	3124[abcd]
Intermediate	None	476	5.65	693[a]	1.44[b]	3095[bcd]
	XAP	473	5.28	681[a]	1.47[b]	3112[abcd]
	XAP + MAN	484	5.48	697[a]	1.44[b]	3114[abcd]
	XAP + ½MAN	483	5.50	691[a]	1.45[b]	3069[cd]
High	None	458	5.51	690[a]	1.45[b]	3121[abcd]
	XAP	479	5.32	676[a]	1.48[b]	3082[bcd]
	XAP + MAN	490	5.32	688[a]	1.46[b]	3060[d]
	XAP + ½MAN	476	5.43	690[a]	1.45[b]	3175[a]
Pooled SEM		11.0	0.110	8.5	0.019	25.3
			P value			
Soy Inclusion		0.10	≤ 0.01	0.05	0.04	0.12
Enzyme		0.48	≤ 0.01	0.12	0.10	0.75
Soy Inclusion x Enzyme		0.38	0.73	0.02	0.01	0.05

*Superscripts designate significance. Treatments denoted by different letters are significantly different at $P \leq 0.05$.

CHAPTER 4. EFFECTS OF A XYLANASE, AMYLASE, AND PROTEASE ENZYME COCKTAIL AND DIRECT-FED MICROBIAL ON BROILER PERFORMANCE AND METABOLIZABLE ENERGY WHEN FED VARIOUS CONCENTRATIONS OF SOY PRODUCTS

Abstract

An experiment was conducted to determine the effects of a xylanase amylase protease (XAP) enzyme cocktail and direct-fed microbial (DFM) on bird performance and metabolizable energy when fed various concentrations of soy products. Dietary treatments contained three different soy (soybean meal 48 and toasted full fat soy) inclusion levels, low soy (20%), intermediate soy (28%), and high soy (35%). All diets were fed from 10 to 21 days of age and were formulated to meet or exceed commercial recommendations for broiler chickens at those ages. Additionally XAP enzyme and DFM product were supplemented across all three soy concentrations generating a 3 x 4 factorial arrangement of treatments. Feed intake, body weight gain (BWG), feed efficiency (FE), and feed conversion ratio (FCR) were measured over the 10 to 21 d period with excreta collected for evaluation of AMEn. Data were analyzed using two-way ANOVA procedure of SAS. Fisher's LSD was used to separate means with an overall $P \leq 0.05$ considered significant. Intermediate soy inclusion with XAP and DFM combination had decreased BWG ($P = 0.02$) compared to other dietary treatments. Feed intake was not significantly different in this experiment. High soy diets had improved FE ($P = 0.02$) and FCR ($P = 0.03$) compared to low and intermediate soy inclusions. Enzyme treatment with XAP only improved FE ($P = 0.04$) and FCR ($P = 0.05$) over XAP and DFM combined supplementation. Low soy diets decreased AMEn ($P \leq 0.01$) compared to intermediate and high soy inclusion levels. Increased fiber from DDGS and canola meal ingredients may explain significantly worse FE, FCR, and AMEn observed in

low soy diets compared to high soy inclusions. Overall, supplementation with XAP and DFM did not improve broiler performance. Supplementing XAP and DFM from hatch or for an increased duration may result in improvements in performance and nutrient digestibility.

Key words: soy, multi-enzyme, direct-fed microbial, broiler

Introduction

Soy is ubiquitous in commercial poultry and other monogastric rations in North America. Soybeans are produced for their high oil yield and meal content, although there are anti-nutritional factors present in these products that may limit performance of poultry. Nutritionally soybean meal is a good source of protein with a fairly well-balanced amino-acid profile, although it is low in methionine, the first limiting amino acid in poultry diets (Centeno et al., 2006). Soy is utilized due to increased lysine content in comparison to other plant protein sources (McNaughton et al., 1981). Although soy is utilized for protein, it is not without limitations. Some of the anti-nutrients present in soy products are haemagglutinins (lectins), saponins, pectins, non-starch polysaccharides (NSP), trypsin inhibitor, phytates, raffinose, stachiose, and β-mannans (Erickson, 1995). Potentially these anti-nutrients can cause damage to the absorptive surface of the gastrointestinal tract of the bird and result in impaired nutrient digestion (Paloheimo et al., 2010). Feed additives are of interest due to their potential to alleviate impacts of anti-nutritional factors on the digestive tract and therefore increase overall nutrient value of ingredients, such as soy used in poultry diets.

Enzyme cocktails like xylanase amylase protease (XAP) primarily target fiber, starch, and protein substrates from plant material (Barletta, 2010). Xylanases function by hydrolyzing NSP present in dietary ingredients and are effective at destroying plant cell wall

structures (Zanella et al. 2004). Additionally, exogenous protease and amylase enzymes increase utilization of proteins in soybean meal, in the upper gastrointestinal tract of broiler chickens, as well as digestion of starch encapsulated in endosperm (Zanella et al., 1999). Use of enzyme supplements designed to degrade insoluble cell wall material can expose starch and protein for digestion, consequently reducing endogenous secretion and potentially decreasing maintenance requirements while increasing nutrient retention (Zanella et al., 1999). Decreasing endogenous secretions can result in lower mucin production and altered microbial populations (Choct and Cadogan, 2001). Protease use in diets containing soybean meal protein break down large storage protein molecules (such as glycinin and β-conglycinin) into smaller fractions which are more easily absorbed by the underdeveloped digestive system of young monogastrics (Bach Knudsen, 1997; Douglas et al., 2000). Also amylase supplementation assists in rapid exposure of starch to digestion in the small intestine, leading to improved growth rates due to heightened nutrient uptake (Zanella et al., 1999; Zanella et al. 2004).

Direct-fed microbial (DFM), or probiotics, are live bacteria marketed as alternatives to antibiotic supplementation. Commonly used bacterial strains in the poultry industry include species of *Bacillus*, *Enterococcus*, *Eshcerichia*, *Lactobacillus*, *Lactococcus*, and *Streptococcus* (Fuller, 2001). Direct-fed microbial can reduce incidence of enteric disease challenge by supporting good microbial populations and improving the health status of an animal by maintaining a beneficial microbial population. Modification of the microbial flora can occur through several mechanisms including competitive exclusion of pathogenic bacteria, regulation of local mucosal cell-mediated immune responses, increasing antibody production, promotion of epithelial barrier integrity, reducing epithelial cell apoptosis,

enhancing dendritic cell induced T cell hypo-responsiveness, improving T cell homing to mesenteric lymph nodes, and augmenting toll-like receptor signaling (Lee et al., 2010).

Research examining the impacts of XAP and DFM has found positive impacts on broiler performance, nutrient digestibility, gut morphometry, and immune parameters (Salminen et al., 1996; Fuller, 2001; Lee et al., 2010). One experiment utilizing an exogenous XAP observed an increase in apparent ileal digestibility (AID) of crude protein (Zanella et al., 1999), while the AID of nitrogen was increased in another experiment (Rutherfurd et al., 2007). Exogenous enzyme supplementation also increased the AID of amino acids in broiler chickens fed corn-soybean meal rations (Cowieson et al., 2010). Others have shown a portion of the improved AID of amino acid stems from reduction of anti-nutritive NSP. It is possible that reduced NSP allows amino acids to interact more closely with enterocytes and therefore increase digestibility (Angkanaporn et al., 1994). Chicks fed a corn-soybean meal diet supplemented with a XAP enzyme cocktail had 1.9% increase in BWG and improved FCR (Zanella et al., 1999). When fed mash diets supplemented with eight differing strains of *B. subtilis* and one multi-strain DFM birds showed no significant alteration in body weight gain (BWG), however significant increases in villus height and crypt depth were observed with DFM treatment, compared to non-supplemented controls. Serum levels of α-1-acid glycoprotein, an indicator of inflammation, were significantly reduced in bird fed diets containing DFM (Lee et al., 2010). Research examining broiler performance when fed corn-soybean meal rations supplemented with DFM have also demonstrated improvement in BWG and FE (Jin et al., 1998; Awad et al., 2009) Data demonstrate the encouraging impacts of DFM or XAP supplementation on nutrient digestibility, performance, and immunity,

although little or no research examining combined supplementation of these feed additives in broilers has been reported.

Diets containing soy products, as the primary protein source, provide substrate for an XAP enzyme cocktail and have also been shown to decrease endogenous secretions (Brenes et al., 1993; Jozefiak et al., 2007; Pirgozliev et al., 2010). Additionally, supplementation with DFM can positively alter microbial populations within the gastrointestinal tract and potentially have positive impacts on bird immunity. Reduction of endogenous secretions has the potential to positively influence broiler immunity and growth performance. Therefore the objective of the current experiment was to determine the effects of the combination of XAP and DFM supplementation on broiler nutrient utilization and growth performance when birds were fed various concentrations of dietary soy.

Materials and Methods

Experimental Design

All animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University before the start of the experiment. In total, 1,152 day old Ross 708 male chicks were obtained from a commercial hatchery (Welp Hatchery, Bancroft, IA) and transported to the Iowa State University Poultry Research and Teaching Unit. The experiment was conducted over two time periods to generate desired replication due to limited cage availability. During the first experimental period four replicates of each diet were performed with eight replicates of each diet housed during experimental period two. Birds were housed in Petersime battery cages (65.8 in^2/ bird) and were exposed to continuous light. Experimental chamber temperature was maintained at 85 ^{o}F, at the start of chick

housing, with a 5 ºF reduction in temperature each week until reaching a final temperature of 70 ºF. All birds were fed a common corn-soybean meal based starter diet, with toasted full fat soybean addition, from 0 to 9 d. On d 10 all birds were sorted into groups by weight to minimize differences in starting cage body weight, wing-banded, individually weighed, assigned to cages, and allotted to experimental treatments. Dietary treatments were randomly assigned to cages. Two experimental diets were formulated to contain high soy, from a combination of toasted full fat soybeans and soybean meal (48%), and low soy, replacing soy products with dried distillers grains with solubles, canola meal, meat and bone meal, and synthetic lysine. Three concentrations of dietary soy inclusion were generated by blending; low (20% soy products: 100% low soy), intermediate (28% soy products: 50-50 mixture of low and high), and high (35% soy products: 100% high soy) (Table 4.1). A 3 x 4 factorial arrangement of treatments was utilized with the three levels of soy (low, intermediate, and high) and XAP and DFM supplementation in four levels (none, XAP, XAP+DFM, and XAP+½DFM). An experimental unit (EU) consisted of a cage containing 8 birds, and there were 12 EU for each of the 12 dietary treatments. Birds were provided *ad-libitum* access to feed and water throughout the experiment and were monitored twice daily with mortality or cull chicks weighed and recorded before disposal. Titanium dioxide was added to all experimental diets at a rate of 0.5% as an inert dietary marker for determination of AMEn. All diets were manufactured at Iowa State University Poultry Research and Teaching Unit and were fed in mash form.

Data Collection

On d 10 and d 21 all birds were individually weighed in order to report BWG for the 10 to 21 d experiment as g/chick. Birds were monitored twice daily with mortality removed from pens as they occurred. Mortality or cull birds were weighed and recorded before disposal. Feed intake was determined for each cage by calculating the difference between the amount of feed offered and the amount of feed refused and reported as kg/cage for the 10 to 21 d period. Chick feed conversion ratio (FCR), defined as grams of feed to grams of BWG, and feed efficiency (FE) which is a ratio of grams of BWG to kilograms of feed consumed. Both were measured from 10 to 21 d using total pen BWG and pen feed intake data, correcting both for any mortality that may have occurred. Fresh and clean excreta samples were collected from pans below each cage from 19 to 21 d for AMEn determination. On d 21 all birds were euthanized via CO_2 to collect ileal contents and leg samples. Both legs were sampled from three randomly selected birds per EU for tibia and toe ash, as well as bone breaking strength determination. The ileum was defined as the segment from the point of Meckel's diverticulum to the ileo-cecal junction. Contents of the ileum were gently expressed with all ileums from each EU combined to generate one sample. Data collected from ileal mineral digestibility and bone ash and breaking will only be presented in the appendix as it does not pertain directly to enzymatic supplementation. All samples were immediately transported to the laboratory and frozen until further analysis.

Laboratory Analysis

Excreta and ileal samples were oven dried for 3 d at 65 °C (Jacobs et al., 2011). Feed samples were also corrected on a dry matter basis by measuring 0.5 g of each sample and

drying them in an oven at 100 ºC for 24 h (Yamato Scientific America Inc., Santa Clara, CA). After drying, excreta and ileal samples were ground through a 1.0 mm screen (Whiley) and feed samples were ground through a 0.5 mm screen (Brinkmann Instruments Inc., Westbury, NY). Feed samples were sent to Experiment Station Chemical Laboratories (University of Missouri, Columbia, MO) for proximate analysis and complete amino acid profile, while ileal samples were analyzed at Central Analytical Laboratory (University of Arkansas, Fayetteville, AR) for calcium and phosphorus ileal digestibility determination using the method published in "Analytical techniques for inorganic contaminants" (AOAC International, 1999). Titanium dioxide concentration was determined (Leone, 1973) for feed and excreta samples based on 0.5% inert marker concentration. Gross energy (GE), for excreta and feed samples, was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Nitrogen concentration of excreta and diet samples was determined using LECO Trumac N (LECO Corp. St. Joseph, MI.). All excreta and diet samples were analyzed in duplicate. The AMEn was calculated using GE, nitrogen content, and titanium dioxide concentration modifying the equation by Scott et al. (1982) to replace chromic oxide with titanium dioxide as a marker. Dietary AMEn values for each treatment were calculated using the following equation.

AMEn = dietary GE - [excreta GE × dietary Ti/excreta Ti - 8.22 × (dietary N - excreta N × dietary Ti/excreta Ti)]

Three entire leg samples from each EU were autoclaved to remove adhering flesh and cartilage caps. Tibias were oven dried at 100 ^{0}C for 24 h and then placed in a furnace at 560 ^{0}C for 24 h to determine bone mineralization (Persia and Saylor, 2006). Middle toes from the three remaining leg samples of each EU were oven dried at 100 ^{0}C for 24 h and then placed in a furnace at 560 ^{0}C for 24 h, with flesh intact, to establish toe mineralization. The same

three remaining leg samples for each EU that were also utilized for toe mineralization, were analyzed to determine bone breaking strength using a Texture Analyzer with texture exponent software. (TA.XTplus, Texture Technologies Corporation, Scarsdale, New York). Flesh remained intact for bone breaking analysis with a three point bend rig set-up using a knife edge blade. Results of calcium and phosphorus digestibility, tibia ash, toe ash, and bone breaking strength are provided in the appendix. Analysis of appendix data were completed as part of another project, but were not included in this thesis work.

Statistical Analysis

Statistical analysis was carried out as a 3 x 4 factorial arrangement of treatments with a completely randomized design. Outliers were removed from data sets prior to analysis. The average of each treatment was calculated and two times the standard deviation of each treatment was added or subtracted from the calculated average. All values falling outside of the calculated range were eliminated. Two-way ANOVA procedure of SAS was used to analyze all treatments (SAS Inst. Inc., Cary, NC). Fisher's LSD was used to separate significant least squares means with probability set at $P \leq 0.05$. Fisher LSD was used only on results with an ANOVA overall $P \leq 0.05$.

Results

In total there were 8 mortalities recorded. Mortalities were spread over five of the twelve treatments, including three separate dietary treatments that had two mortalities apiece and two others that had a single mortality during the 10 to 21 d experimental period. The rate of mortality was fairly equal among dietary treatments and no discernible patterns were

noted. An interaction was observed between soy inclusion and feed additive for bird BWG, in that the intermediate soy inclusion supplemented with XAP+DFM resulted in lower BWG (P = 0.02) compared to all other treatments (Table 4.2). Feed intake was not significantly altered by soy inclusion or feed additive treatment. Main effects were seen for both soy inclusion and feed additive on FE and FCR in this experiment. High soy inclusion improved FE (P = 0.02) (887, 872, 866 g/kg) and FCR (P = 0.03) (1.13, 1.15, 1.15 g/g) compared to both intermediate and low soy treatments. Feed efficiency for the XAP+DFM combination was decreased (P = 0.04) while FCR was increased (P = 0.05) compared to XAP supplementation alone, regardless of soy inclusion. There was a significant main effect of soy inclusion on AMEn ($P \leq 0.01$). Nitrogen corrected apparent metabolizable energy of low soy diets was decreased ($P \leq 0.01$) compared to intermediate and high soy inclusions (3127, 3198, 3194 kcal/kg, respectively).

Discussion

Direct-fed microbials have been shown to benefit broiler production through regulating microbial homeostasis in the intestine in combination with maintaining barrier function of the gastrointestinal system (Salminen et al., 1996). It has been reported that DFM supplementation could potentially positively affect energy digestibility of poultry by improving intestinal integrity (Fuller, 2001; Lee et al., 2010). Another proposed mode of action is through competitive exclusion of pathogenic bacteria (Nurmi and Rantala, 1973; Schneitz, 2005), which has the potential to improve bird performance (Corrier et al., 1995) through improved immune function (Schneitz, 2005). With these concepts in mind, the hypothesis was that the combination of XAP and DFM would result in increased

performance and energy digestibility as breakdown products of XAP supplementation have the opportunity to possibly serve as prebiotics for DFM treatments.

In the current experiment, supplementation of XAP or the combination of XAP and DFM did not increase BWG in comparison to the non-supplemented control diets. In fact the XAP with the highest concentration of DFM decreased BWG in the intermediate soy diet. One explanation for this response could be that in the low stress condition of a battery cage, the higher concentration of DFM is actually increasing maintenance requirements of the birds by priming the immune system at the cost of performance, but this does not explain why it was only noted in the intermediate soy concentration and not across all soy concentrations. In the battery cage, environmental and sanitary conditions were well controlled and therefore pathogenic or general microbial challenge to the birds system may have been relatively low. Therefore DFM supplementation at the higher concentration may have unnecessarily increased energy expenditure towards maintenance of the intestinal epithelium resulting in decreased energy or nutrients available for bird growth (Salminen et al., 1996).

Experimental diets were formulated based on the concept that anti-nutritional factors present in soy products have the potential to limit bird growth, feed efficiency and energy digestibility as soy concentration increases. However in this experiment, high soy inclusion diets improved both FE and FCR compared to intermediate and low soy treatments. Anti-nutritional components of high soy diets did not seem to limit these performance parameters as speculated. One explanation could be that low and intermediate soy diets were formulated with increased concentrations of canola meal and dried distillers grains with solubles (DDGS) as a replacement for soy products in the high soy diets. Canola meal and DDGS ingredients both have increased fiber concentrations compared to corn and soy ingredients

(Table 4.1). Poultry are inefficient in utilizing fiber due to shorter digesta passage time and therefore very limited fermentation capacity (Choct and Cadogan, 2001). Addition of lower fiber and higher digestible ingredients by increasing soy concentration may decrease the opportunity to consistently demonstrate the negative effects of soy anti-nutrient inclusion on broiler performance and energy digestibility.

Feed efficiency for the XAP+DFM treatments was decreased and FCR was increased compared to XAP supplementation alone, although neither was different from the non-supplemented and XAP+½DFM treatments. Exogenous enzymes have been shown to allow the birds to access nutrients that endogenous enzymes alone cannot (Barletta, 2010). Supplementation of corn-soybean meal diets with exogenous enzymes has previously resulted in increased digestibility of nutrients (Zanella et al., 1999; Rutherfurd et al., 2007; Cowieson et al., 2010), and improved performance (Zanella et al., 1999; Zanella et al. 2004). Addition of DFM alone has been shown to increase FE in broilers fed corn-SBM diets (Jin et al., 1998, Mountzouris et al., 2010). Other research has indicated BWG and FE were significantly increased for broiler chicks fed DFM for a 45 d period (Awad et al., 2009). However in this experiment addition of XAP and DFM to broiler diets did not result in improved FE and FCR performance. Again, the FE and FCR response may be due to XAP and DFM potentially stimulating the immune system in a low stress model resulting in reduced efficiency. Stimulation of the immune system, in a low stress environment, would result in unnecessary energy expenditure and reduced energy available for bird performance (Salminen et al., 1996)

Nitrogen corrected apparent metabolizable energy of low soy diets was decreased compared to intermediate and high soy inclusions. No significant response for feed additive

treatment was observed for AMEn, in accordance with the results noted for FE and FCR, as feed additive treatments were not different from non-supplemented controls. Increased fiber content of the low soy diet from canola meal and DDGS ingredients might cause reduced digestibility resulting in decreased energy availability (Choct and Cadogan, 2001).

Some researchers have shown the positive impacts of feeding DFM or XAP alone on broiler AMEn, although results varied with supplementation for different periods of time. Previous research indicates supplementation of DFM in corn-soybean meal diets fed to broilers from 1 to 42 d increased AMEn (Li et al., 2008). Other research supplementing XAP from 8 to 21 d found no effect on AMEn during the trial period (Douglas et al., 2000). Although there is limited published research that has evaluated this response, it is possible that feed additives fed for a longer duration or supplemented from day of hatch would result in a greater and more consistent response in dietary AMEn and broiler growth performance. Responses to supplementation of XAP and DFM at day of hatch may be affected by altering the naive microbial population and reducing gastrointestinal maintenance requirements later in the broiler life cycle.

As noted, the evaluation of multiple feed additives and dietary soy concentration is complex. Previous research examining the effects of supplementing XAP and DFM alone indicate these two feed additives could potentially work synergistically. Direct-fed microbials have been shown to improve integrity of the gastrointestinal tract (Salminen et al., 1996; Fuller, 2001; Lee et al., 2010) while complementing the microbial population (Salminen et al., 1996) potentially allowing for improved efficiency of the host relationship with the microbial flora and with nutrients released from plant cell walls by exogenous XAP (Joergensen et al., 1996). In the absence of environmental and immune stressors the

supplementation of DFM may reduce energy available for broiler growth performance by unnecessarily increasing energy utilization of the gastrointestinal tract epithelium (Salminen et al., 1996). In addition time of first feed additive addition is important as supplementation of DFM from day of hatch may reduce energy expenditure for gastrointestinal maintenance as the host microbial populations become established. During transitions and throughout epithelial maturation the epithelial integrity and microbial population must be maintained which requires energy expenditure (Batal and Parsons, 2003). Although the results of the current research were inconclusive, several factors were discussed that should be considered when evaluating the effects of multiple feed additives on diets that contain various concentrations of soybean products.

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Table 4.1. Composition of broiler starter and experimental diets fed from 0 to 9 d and 10 to 21 d, respectively. Soy concentrations for experimental diets were low (20%), intermediate (28%), and high (35%). xylanase, amylase, and protease enzyme cocktail and direct-fed microbial were added to generate final experimental diets.

Diet	Starter	Low Soy	Intermediate Soy	High Soy
Ingredient Composition	----------(%)----------			
Corn	51.83	51.85	51.18	50.52
Soybean meal (48% CP)	23.38	20.00	22.50	25.00
Soybeans (toasted full fat)	5.00	0.00	5.00	10.00
Dried distillers grains with solubles	10.00	15.00	12.48	9.95
Meat/bone meal	4.13	2.90	1.45	0.00
Canola meal	2.39	5.47	2.73	0.00
Soy oil	0.50	1.82	1.16	0.50
Salt	0.31	0.30	0.35	0.39
DL-methionine	0.29	0.20	0.21	0.22
L-threonine	0.05	0.00	0.01	0.02
Bio-Lys[1]	0.47	0.40	0.30	0.21
Limestone	0.92	0.84	0.97	1.11
Di-calcium phosphate	0.00	0.00	0.43	0.86
Choline chloride	0.10	0.10	0.10	0.10
Vitamin and mineral premix[3]	0.63	0.63	0.63	0.63
Phytase[2]	0.00125	0.00125	0.00125	0.00125
Titanium dioxide	0.00	0.50	0.50	0.50
Chemical Composition (Calculated)	----------(%)----------			
Crude protein	23.45	21.75	22.25	22.75
ME (kcal/kg)	2960	2960	2960	2960
Calcium	0.89	0.74	0.74	0.74
Non-phytate phosphorus	0.35	0.30	0.30	0.30
Total Fat	5.07	5.78	5.64	5.49
Total Fiber	3.38	3.68	3.49	3.31
Digestible Methionine	0.60	0.50	0.51	0.52
Digestible Lysine	1.27	1.10	1.13	1.15
Digestible Threonine	0.83	0.73	0.77	0.80
Digestible Methionine+Cysteine	0.94	0.84	0.85	0.86
Chemical Composition (Analyzed)	----------(%)----------			
Crude protein		21.96	22.49	22.95
Fat		5.88	5.31	4.54
Fiber		3.42	3.17	3.13
Methionine		0.55	0.55	0.51
Lysine		1.30	1.29	1.23
Threonine		0.79	0.83	0.82
Methionine+Cysteine		0.87	0.88	0.82

[1,2,3] Additional information provided in Appendix C.

Table 4.2. Effects of soy inclusion along with xylanase, amylase, and protease (XAP) and direct-fed microbial (DFM) treatment on nitrogen corrected apparent metabolizable energy (AMEn), body weight gain (BWG), feed intake (FI), feed efficiency (FE), and feed conversion ratio (FCR) of 10 to 21 d old broilers.

Soy Inclusion	Feed Additive	BWG (g/ck)	FI (kg/pen)	FE (g/kg)	FCR (g/g)	AMEn (kcal/kg)
Low		453	5.57	869 [b]	1.15 [a]	3127 [b]
Intermediate		451	5.54	872 [b]	1.15 [a]	3198 [a]
High		453	5.45	887 [a]	1.13 [b]	3194 [a]
Pooled SEM		4.3	0.044	5.0	0.007	14.1
	None	452	5.52	876 [ab]	1.14 [ab]	3150
	XAP	455	5.49	887 [a]	1.13 [b]	3183
	XAP + DFM	446	5.57	863 [b]	1.16 [a]	3190
	XAP + ½DFM	455	5.50	877 [ab]	1.14 [ab]	3170
Pooled SEM		5.0	0.050	5.8	0.008	16.6
Low	None	445 [ab]	5.50	865	1.16	3115
	XAP	466 [a]	5.62	879	1.14	3151
	XAP + DFM	451 [a]	5.64	861	1.16	3129
	XAP + ½DFM	450 [a]	5.53	870	1.15	3113
Intermediate	None	465 [a]	5.69	883	1.13	3143
	XAP	455 [a]	5.46	893	1.12	3216
	XAP + DFM	425 [b]	5.47	845	1.19	3219
	XAP + ½DFM	457 [a]	5.53	865	1.16	3214
High	None	447 [ab]	5.37	881	1.14	3192
	XAP	445 [ab]	5.39	889	1.13	3181
	XAP + DFM	463 [a]	5.58	884	1.13	3221
	XAP + ½DFM	457 [a]	5.44	895	1.12	3182
Pooled SEM		8.7	0.087	10.0	0.014	28.2
		P value				
Soy Inclusion		0.90	0.12	0.02	0.03	≤ 0.01
Feed Additive		0.56	0.72	0.04	0.05	0.35
Soy Inclusion x Feed Additive		0.02	0.28	0.28	0.25	0.67

*Superscripts designate significance. Treatments denoted by different letters are significantly different at $P \leq 0.05$.

CHAPTER 5. CONCLUSIONS

Soy products are ubiquitous in poultry rations due to their well balanced amino acid profile, and further processing of soybeans can produce a high quality protein source. Although soybean meal and other soy products are considered quality feed ingredients, the presence of anti-nutritional components in soy is a concern and a lost opportunity to poultry producers. Some of the anti-nutrients in soy are NSP, β-mannans, trypsin inhibitor, lectins, saponins, and pectins (Marsman et al., 1997; Kocher et al., 2003). Soy anti-nutrients pose a threat to nutrient digestibility and proper physiological responses and therefore bird growth performance and feed efficiency. Several feed additives have been developed to try to address this opportunity to improve the utilization of soy products in poultry diets by increasing energy availability.

Exogenous enzymes and direct-fed microbials show potential in addressing the complex issue of anti-nutrients present in soy products and subsequent energy utilization by the bird. Published literature on the effects of multi-enzyme combinations and direct-fed microbial (DFM) impacts on soy product utilization is limited. Therefore the objective of this thesis research is to examine the effects of feeding a multi-enzyme (enzyme product containing xylanase, amylase, and protease activity: XAP) in combination with either a β-mannanase (MAN) or DFM on broiler growth performance and energy digestibility when supplemented into diets that contain three concentrations of dietary soy product inclusions, from both soybean meal and toasted full fat soy ingredients.

The hypothesis for the first experiment was that a combination of exogenous enzymes including XAP and MAN would result in an increase in weight gain or feed efficiency and an increase in energy digestibility that would be more pronounced in a diet that contains higher

concentrations of higher anti-nutrient soy products by direct degradation of some of the anti-nutrients. Both xylanase and β-mannanase enzymes are targeting different cell wall NSP, not only increasing access to cell contents but possibly releasing energy directly from sugar release by NSP hydrolysis (Smits and Annison, 1996). Amylase and protease exogenous enzymes are capable of degrading starch and protein substrates released from increased cell wall degradation by exogenous xylanase and β-mannanase enzymes (Bedford, 1996). In addition to effects on NSP degradability, inclusion of MAN has also been shown to modulate the function of the broiler immune system (Zou et al., 2006). The hypothesis of the experiment was that high soy diets would result in the lowest performance, but in fact, the diet that contained the lowest soy concentration resulted in the highest feed intake. Low soy diets contained increased concentrations of canola meal and DDGS compared to intermediate and high soy treatments. Fiber is a large component of canola meal and DDGS and is not efficiently utilized by broilers due to reduced gastrointestinal tract length and fermentation capabilities of broilers compared to other monogastric species (Choct and Cadogan, 2001).

In experiment one the addition of exogenous XAP and MAN did not drive weight gain, but did increase the efficiency in which broiler chicks processed feed into weight gain resulting in increased broiler performance in the low soybean meal fed birds. Keeping in mind that the low soy diets resulted in reduced performance in this experiment, it is not surprising that the enzyme combinations resulted in increased performance in the low soy diets. Although there was an interaction with AMEn at the end of the experimental period this interaction was not consistent with increased feed efficiency in the low soy diets suggesting that the positive effects of enzyme combinations are due to factors not associated with increased energy digestibility. Other possible mechanisms could include increased

protein or amino acid utilization driving feed efficiency or a possible change in chick maintenance requirements (Bedford, 1996; Kocher et al., 2003).

The hypothesis of the second experiment was that supplementation of XAP and DFM would be complementary, possibly resulting in increased nutrient digestibility and broiler growth performance, these effects being most pronounced in high soy diets with increased anti-nutrient content. The use of XAP has been shown to increase nutrients and energy available to the bird for growth (Bedford 1996; Zanella et al., 2004) and potentially can result in degradation of anti-nutrients present in soy products (Kocher et al., 2003). Inclusion of a DFM increases the beneficial microbial population in the gastrointestinal tract and competitively excludes pathogenic organisms, therefore leading to increased immune stimulation (Jackson et al., 2004). Increased immune stimulation could decrease the energy required by the bird for maintenance, however in the absence of disease challenge increased immune function could reduce the energy available for growth. Another potential benefit of feeding XAP and DFM in combination is that the breakdown products of XAP supplementation may act as a prebiotic source providing energy for the DFM (Marsan et al., 1997; Lee et al., 2010). The combination of XAP and MAN or XAP and DFM has potential to increase bird growth performance, nutrient digestibility, feed efficiency, and immune function when supplemented in soy protein diets.

Results from experiment two did not show the growth stimulating effects that had been anticipated with the XAP and DFM combination. XAP and DFM supplementation with intermediate soy inclusion resulted in significantly reduced BWG compared to all other treatments. During the transition from a starter diet, absent of feed additives, to a grower diet supplemented with XAP and DFM, potential fluctuations in the number and population of

protozoa and bacteria in the intestine may result (Choct and Annison, 1990; Choct and Cadogan, 2001), along with physiological changes in the intestinal epithelium (Batal and Parsons, 2003). These changes could cause an increase in host endogenous secretions and energy funneled from the diet in order to effectively make a dietary transition (Ide et al., 1989). Reduced energy available to the birds may have been due to an increase in high fiber ingredients such as canola meal and DDGS (NRC, 1994). Low soy diets had significantly reduced AMEn and the decreased metabolizable energy available from high fiber ingredients present in lower soy rations may have reduced bird growth performance.

Supplementation with XAP+DFM resulted in significantly worse FE and FCR compared to XAP supplementation alone in experiment two, although neither were different from non-supplemented treatments. In this case it was not advantageous to provide supplementation of DFM in combination with XAP. During the initial 0 to 9 d pre-trial period bird microbial flora may have adapted to ingredients along with toasted full fat soybeans in the starter diet. Feed additives were not included in starter diets and therefore the microbial population was not able to adapt to these additives until day 10 (Choct and Annison, 1990; Choct and Cadogan, 2001). Potentially when adaptation did occur energy may have been expended in order to effectively make a transition to the experimental diet. The gastrointestinal tract may have been able to efficiently support the transition to XAP supplementation. However additional inclusion of DFM in combination with XAP might have expended more energy and exceeded the gastrointestinal capabilities to efficiently support the microbial population and gastrointestinal health during the short 10 to 21 d period. There may be another possible explanation for decreased efficiency and growth performance with additional supplementation of DFM. Since environmental conditions, both

climate and sanitation, were well controlled in the research setting little immune challenge would be present. Without immune compromising conditions supplementation of DFM may not be of direct benefit and instead unnecessarily expend energy for maintenance of the immune system and management of the microbial population rather than directing energy toward bird growth performance (Lee et al., 2010).

Since experiment two utilized an XAP and DFM rather than the combination of XAP and MAN, birds supplemented with both exogenous enzymes may have had increased energy available compared to the single enzyme treatment. Increased nutrient liberation from the XAP and MAN combination compared to XAP and DFM could result in increased available energy and therefore decreased feed consumption. This may be because DFM are not degrading substrates to use as a potential energy source like XAP and MAN enzymes. Also the DFM may have used energy that the bird could have otherwise utilized for maintenance and growth.

The negative effects of the low soy diet manifested in various ways over both experiments. In general, high soy diets outperformed other soy inclusions in relation to FE, FCR, and AMEn. Since low soy diets had decreased AMEn birds required increased intake to make up for this energy difference between low soy and higher soy inclusions. Non-supplemented treatments in experiment one resulted in significantly increased feed intake compared to those with XAP and MAN added. This could indicate that enzyme supplementation increased the efficiency that the birds utilized dietary energy. Therefore addition of multi-enzyme combinations can provide benefits to bird efficiency and feed consumption. Another important consideration is adding feed additives at day of hatch rather than on d 10, allow a longer adaptation period to the combination of XAP+DFM and

XAP+MAN resulting in potential for more consistent differences in bird growth performance. High soy diets with XAP+MAN supplementation were significantly decreased compared to high soy diets with XAP+½MAN, in regards to AMEn. Increased concentrations of carbohydrase enzyme may have energy depressing effects, although the impacts on AMEn in relation to multi-enzyme combinations have been sparsely examined (Daskiran et al., 2004). Low soy inclusion treatments reduced AMEn in experiment two, possibly due to increase in high fiber ingredients such as DDGS and canola meal. Even though high soy inclusion increased AMEn in experiment two, results for experiment one demonstrate the potential energy depressing impacts of additional carbohydrase addition to high soy rations.

Overall, high soy inclusion diets out performed low soy treatments. This might be attributed to the increased fiber ingredients included in low soy diets. Exogenous XAP and MAN enzymes are used to target mainly NSP anti-nutrients and therefore may not have direct benefit on utilization of DDGS and canola meal ingredients. Enzyme supplementation did result in improved nutrient digestibility of high soy experimental diets. Exogenous enzymes such as XAP and MAN target cell wall NSP and other substrates making nutrients available to for energy metabolism while DFM complements the microbial flora and stimulates the immune system. Additional inclusion of DFM in combination with XAP supplementation did not result in improved broiler performance. These results suggest supplementation of exogenous enzymes and DFM from day of hatch may improve the benefit of such feed additives on increasing efficiency of nutrient utilization. Utilization of these feed additives from day of hatch may also provide gut health and immune benefit for young birds or in immune challenge situations. Supplementation of high soy diets for broiler

chickens with carbohydrase enzymes might have potential to improve overall bird performance by increasing energy availability when supplemented on day of hatch. When formulating low soy rations increased fiber concentration should be taken into consideration and avoided if feasible.

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APPENDIX A. SUPPLEMENTAL DATA EXPERIMENT 1

Effects of xylanase, amylase, and protease enzyme cocktail in combination with β-mannanase enzyme in diets containing variable soy inclusion on tibia ash, tibia breaking strength, toe ash, ileal Ca, and P digestibility of 10 to 21 d old broilers.

		Tibia			Ca	P
Soy Inclusion	Enzyme	Ash	Breaking	Toe Ash	digestibility	digestibility
		(%)	(g)	(%)	(%)	(%)
Low		44.8[b]	13082	12.24	34.4	53.6
Intermediate		45.2[ab]	13244	12.19	34.0	50.1
High		45.9[a]	12605	12.25	38.6	55.7
Pooled SEM		0.26	293	0.116	1.4	1.01
	None	45.2	13622	12.33	35.4	54.3
	XAP	45.4	12927	12.01	36.6	54.0
	XAP + HC	45.7	12871	12.42	32.0	51.5
	XAP + ½HC	45.1	12487	12.12	38.7	52.8
Pooled SEM		0.30	338	0.134	1.7	1.16
Low	None	44.6	12803	12.27	34.9	58.5[a]
	XAP	45.1	13775	12.26	33.8	53.3[abc]
	XAP + HC	45.3	13058	12.38	30.8	51.2[bcd]
	XAP + ½HC	44.4	12692	12.03	38.0	56.4[ab]
Intermediate	None	45.1	14497	12.19	32.6	54.1[abc]
	XAP	45.2	12453	12.29	38.6	55.4[abc]
	XAP + HC	45.5	13132	12.45	30.2	50.4[cd]
	XAP + ½HC	45.3	12894	11.81	34.6	46.4[d]
High	None	45.9	13567	12.54	37.0	55.4[abc]
	XAP	45.7	12554	12.61	33.5	54.9[abc]
	XAP + HC	46.2	12424	12.44	36.6	57.1[a]
	XAP + ½HC	45.6	11876	12.51	43.4	57.0[a]
Pooled SEM		0.52	586	0.232	2.9	2.01
		P value				
Soy Inclusion		0.01	0.22	0.09	0.11	≤ 0.01
Enzyme		0.53	0.18	0.37	0.10	0.19
Soy Inclusion x Enzyme		0.99	0.26	0.87	0.81	≤ 0.01

APPENDIX B. SUPPLEMENTAL DATA EXPERIMENT 2

Effects of xylanase, amylase, and protease enzyme cocktail in combination with direct-fed microbial in diets containing variable soy inclusion on tibia ash, tibia breaking strength, toe ash, ileal Ca, and P digestibility of 10 to 21 d old broilers.

		Tibia			Ca	P
Soy Inclusion	Enzyme	Ash	Breaking	Toe Ash	digestibility	digestibility
		(%)	(g)	(%)	(%)	(%)
Low		46.5	13007	12.9	32.1	54.0
Intermediate		46.7	12880	13.0	29.1	55.1
High		46.7	12268	13.1	33.9	59.1
Pooled SEM		0.19	237.8	0.08	1.49	0.93
	None	46.7	12793	13.1	27.8^{b}	50.8
	XAP	46.5	12890	12.9	33.9^{a}	56.0
	XAP + DFM	46.8	12331	13.0	30.7^{ab}	57.4
	XAP + ½DFM	46.6	12881	13.1	34.3^{a}	59.8
Pooled SEM		0.22	274.6	0.09	1.72	1.08
Low	None	46.7	12855	12.9	27.7	48.5^{d}
	XAP	46.4	13440	12.8	31.8	50.4^{cd}
	XAP + DFM	46.9	12509	12.9	32.6	57.5^{ab}
	XAP + ½DFM	46.2	13223	13.1	36.1	59.4^{ab}
Intermediate	None	46.8	13830	13.26	25.5	46.5^{d}
	XAP	46.6	12884	12.9	30.9	60.5^{ab}
	XAP + DFM	46.6	12403	12.9	28.2	55.5^{bc}
	XAP + ½DFM	46.9	12404	13.0	31.7	58.0^{ab}
High	None	46.8	11687	13.0	30.2	58.0^{ab}
	XAP	46.6	12297	12.9	39.1	57.2^{ab}
	XAP + DFM	46.9	12073	13.1	31.3	59.3^{ab}
	XAP + ½DFM	46.6	13016	13.2	35.1	61.8^{a}
Pooled SEM		0.39	475.6	0.16	2.99	56.25
Soy Inclusion		0.69	0.07	0.53	0.08	≤ 0.01
Enzyme		0.77	0.44	0.28	0.03	≤ 0.01
Soy Inclusion x Enzyme		0.94	0.16	0.75	0.83	≤ 0.01

APPENDIX C: EXPERIMENT 1 AND 2 DIET FORMULATION SUPERSCRIPTS

[1]Contains 50.7% of l-Lys in the form of l-Lys sulfate.

[2]Contained 500 FTU/kg phytase enzyme activity.

[3]Provided per kg of diet: Selenium-200 μg; Vitamin A-6,600 IU; Vitamin D3-2,200 IU; Vitamin E-14.3 IU; Menadione-880 μg; Vitamin B12-9.4 μg; Biotin-33 μg; Choline-358 mg; Folic acid-1.1 mg; Niacin-33 mg; Pantothenic acid-8.8 mg; Pyridoxine-880 μg; Riboflavin-4.4 mg; Thiamine-1.1 mg; Iron- 226 mg; Magnesium-100 mg; Manganese-220 mg; Zinc-220 mg; Copper-22 mg; Iodine- 675 μg.